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Full Length Research Paper

Extraction, partial purification and characterization of pectinases isolated from *Aspergillus species* cultured on mango (*Mangifera indica*) peels

Daniel Ikenna UDENWOBELE[1]*, Chukwunonso Anthony NSUDE[1], Arinze Linus EZUGWU[1], Sabinus Oscar Onyebuchi EZE[1], Chukwudi ANYAWU[2], Peter Nzemndu UZOEGWU[3] and Ferdinand Chiemeka CHILAKA[1]

[1]Enzymology and Protein Chemistry Unit, Department of Biochemistry, University of Nigeria, Nsukka, Enugu, Enugu State, Nigeria.
[2]Department of Microbiology, University of Nigeria, Nsukka, Enugu, Enugu State, Nigeria.
[3]Department of Biochemistry, University of Nigeria, Nsukka, Enugu, Enugu State, Nigeria.

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Pectinase was produced from a culture of *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus.* Pectinase synthesis was achieved using mango (*Mangifera indica*) pectin extract as an inducer during pectinolytic fungi isolation while submerged fermentation process was carried out using ground mango peels as the sole carbon source. Substrate fermentation was evaluated within seven days by monitoring the pectinase activity every 24 h. The highest pectinase secretion was obtained from *A. niger* and *A. fumigatus* after 92 h (day 4) of incubation, while in *A. flavus,* it was after 120 h (day 5). Crude enzyme extracts from the three organisms were partially purified by a combination of ammonium sulphate precipitation and dialysis with an approximately two-fold purification of the pectinase and a yield of 5.4, 7.66 and 5.99% for *A. niger, A. fumigatus* and *A. flavus*, respectively after dialysis. The specific activities of 1.62, 1.79 and 1.86 U/mg for *A. niger, A. fumigatus* and *A. flavus* enzymes were calculated, respectively. Pectinase from *A. niger* and *A. fumigatus* had pH and temperature optima of 5.0 and 40°C, respectively, while that from *A. flavus* had pH and temperature optima of 5.0 and 45°C. The Michealis constant, K_m and the maximum velocity, V_{max} determined from Lineweaver-Burk plots of initial velocity data at different concentrations of the mango pectin extract were 0.357 mg/ml and 35.34 U; 0.156 mg/ml and 68.0 U; and 0.261 mg/ml and 60.61 U; for the enzymes from *A. niger*, *A. fumigatus* and *A. flavus,* respectively. The results suggest that mango peels can be used for value added synthesis of pectinase, an important enzyme with numerous biotechnological applications.

Key words: Mango peels, mango pectin extract, pectinase, *Aspergillus niger*, *Aspergillus fumigatus, Aspergillus flavus*, submerged fermentation, partial purification, characterization.

INTRODUCTION

In the processing and utilization of mango fruit in products such as juices, nectars concentrates, jams, jelly powders and flakes, wastes are generated in the form of peels and kernels. According to Larrauri et al. (1996), by-products of industrial mango processing may amount to 35 to 60% of the total fruit weight. These by-products

represent a serious disposal problem and ways for a sustainable agricultural production have to be discovered. Mango kernels have been utilized as source of oil (Moharram and Moustafa, 1982), natural antioxidants (Puravankara et al., 2000), starch (Kaur et al., 2004), flour (Arogba, 2002) and feed (Ravindran and Sivakanesan, 1996). Mango peels can be utilized in the production of value added products such as biogas, pectin peel oil, dietary fiber and predominantly pectinases that can be easily harnessed. Among these products, pectin and pectinases have a wide global market (Bali, 2003). Pectins are complex and structural polysaccharides found in the primary cell wall and middle lamella of fruits and vegetables where they function as hydrating agent and cementing material of the cellulosic network (Jarvis et al., 2003; Favela-Torres et al., 2006). Pectic polysaccharides such as homogalacturonan (HGA), xylogalacturonan (XGA), apiogalacturonan, rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII) have been isolated from plant primary cell wall (Harholt et al., 2010). Pectinases are a group of enzymes that catalyze the breakdown of pectins. They are classified into protopectinases, esterases and depolymerases based on their preferred substrate, the degradation mechanism and the type of cleavage (Jayani et al., 2005). Submerged (SMF) and solid state fermentation (SSF) processes have been widely used for pectinase production by different organisms. However, microbial pectinases are produced mostly by SMF in a process that is influenced by the type and concentration of the carbon source, the culture pH, the incubation temperature and the oxygen concentration (Rashmi et al., 2008).

MATERIALS AND METHODS

3,5-Dinitrosalicylic acid (DNS) was a product of Sigma chemical company (USA). All other chemicals used in this work were of analytical grade and were products of Merck (Germany), BDH chemical limited (England), May and Baker limited (England).

Collection of mango fruits

Mature mango fruits (*Mangifera indica)* were collected from the major markets in Amechi Awkunanaw in Enugu State, Nigeria.

Ground mango peels

The fruit peels were peeled, cut into pieces and washed with 96% ethanol to disinfect the peels. The washed peels were then sun dried for seven days and ground into powder.

Extraction of pectin

Pectin was extracted using the method described by McCready (1970) and the yield determined by the equation:

$$\text{Percentage yield} = \frac{\text{mass of pectin extracted}}{\text{mass of ground mango peels}} x\ 100$$

Isolation of microorganism

Three *Aspergillus* strains were isolated from soil of decaying mango fruits and vegetables located in the University of Nigeria Nsukka Campus Enugu State, Nigeria using the method described by Martin et al. (2004). The soil samples were collected in clean dry plastic containers and transported to the laboratory.

Fungal identification

Three days old pure culture of *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus,* were used in preparing microscopic slides. A clump of the mycelia was dropped on the slide and a drop of lactophenol blue was added to it. Identification was carried out using 400 magnifications according to the method of Barnett and Hunter (1972). Species identification was performed by examining both macroscopic and microscopic features of a three day old pure culture. Colour, texture, nature of mycelia and/or spores produced, growth pattern in addition to microscopic features such as separation, spore shapes and so on were examined. The spores of the three *Aspergillus* strains were grown and maintained on potato dextrose agar.

Pectinase production

Pectinase was produced by submerged fermentation according to the method of Martin et al. (2004). Submerged fermentation (SmF) technique was employed using 21 250 ml Erlenmeyer flask containing 100 ml of sterile cultivation medium optimized for pectinase with 0.1% NH_4NO_3, 0.1% $NH_4H_2PO_4$, 0.1% $MgS0_4 \times 7H_2O$ and 1% of ground mango peel. The flask was stoppered with aluminium foil and autoclaved at 121°C for 15 min to sterilize the culture medium. Three days old cultures were used to inoculate the flasks. In every sterile flask, two discs of the respective fungal isolates were added using a cork borer of diameter 10 mm. The culture was incubated for seven days at room temperature (30°C). The fermentation media was agitated at 250 rpm on rotary shaker. At each day of harvest, flasks were selected from the respective groups and mycelia biomass separated by filtration through Whatman filter paper No. 1. The filtrate for each day was assayed for pectinase activity till the 7th day of fermentation. After the seven days pilot SmF studies, the day of peak pectinase activity was chosen for mass production of enzyme from the respective fungal isolates. Several (21) 250 ml Erlenmeyer flasks were used to produce 3.0 L of the enzyme. Harvesting was carried out on the respective peak days of enzyme activity. The harvested 3.0 L of the enzyme was used as crude enzyme.

Pectinase assay

Pectinase activity was evaluated by assaying for polygalacturonase

*Corresponding author. E-mail: daniel.udenwobele@unn.edu.ng. Tel: +2347031524415.

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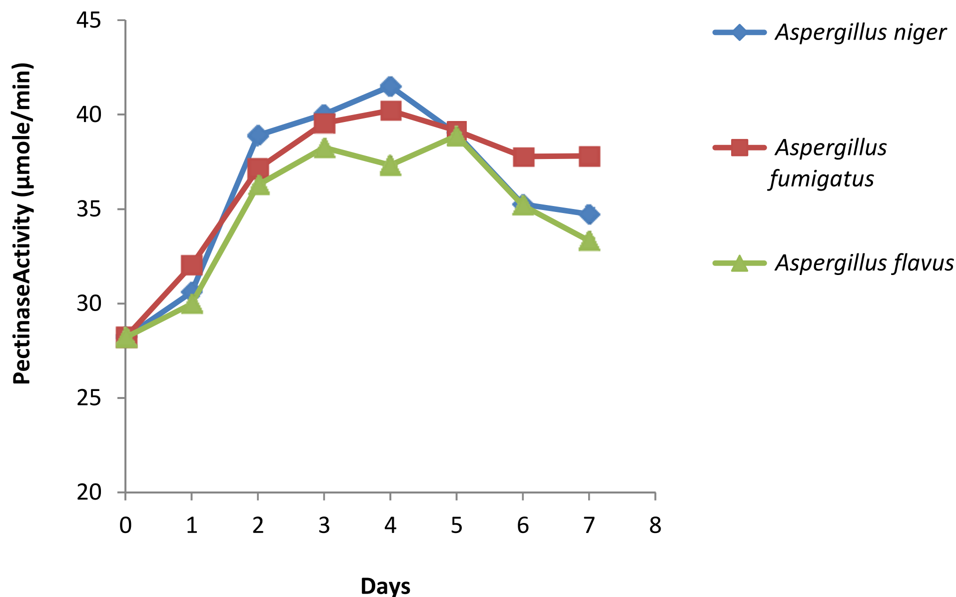

Figure 1. Determination of duration for peak pectinase production from *Aspergillus* species.

(Pg) activity of the enzyme. This was achieved by measuring the release of reducing groups from mango pectin using a modification of the 3,5-dinitrosalicylic acid (DNS) reagent assay method described by Miller (1959) as described in Wang et al. (1997) with little modifications. The reaction mixture containing 0.5 ml of 0.5% mango pectin in 0.05 M sodium acetate buffer of pH 5.0 and 0.5 ml of enzyme solution were incubated for 1 h. 1 ml of DNS reagent was added and the reaction was stopped by boiling the mixture in a boiling water bath for 10 min. The mixture volume was made up to 4 ml with 1 ml of Rochelle salt solution and 1 ml of distilled water. The reaction mixture was allowed to cool and then absorbance was read at 575 nm. The standard curve was prepared for reducing groups with galacturonic acid. One enzyme unit of pectinase is the number of reducing groups measured in terms of galacturonic acid, produced as a result of the action of 0.5 ml of enzyme extract in 1 min at 30°C.

Protein was determined by the method of Lowry et al. (1951). Specific activity is the ratio of the total activity to total protein.

Enzyme purification

The crude enzyme was brought to 80% ammonium sulphate saturation and then dialysed. Dialysis tubes preserved in 90% ethanol were rinsed several times with distilled water and then buffered till they were clean of the ethanol. 0.05 M sodium acetate buffer pH 5.0 was used for enzyme dialysis. Dialysis was carried out for 14 h with continuous stirring and buffer changed every 6 h aiming to remove low molecular weight substances and other ions that may interfere with enzyme activity. After dialysis was complete, the partially purified enzyme was stored frozen at -24°C.

Enzyme characterization

The effects of pH and temperature were determined according the method described by Miller (1959) as described in Wang et al. (1997) with little modifications as described previously under pectinase assay. The kinetic parameters were calculated from Lineweaver-Burk plots of initial velocity data at different concentrations of mango pectin extract (0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/ml).

RESULTS

All the experiments were conducted in triplicate and the result is the mean of the data derived. Three strains of pectinolytic fungi (*A. niger, A. fumigatus* and *A. flavus)* were isolated from soil of decaying fruits and vegetables. Pectin was extracted from mango peel with a yield of 15.2% at pH of 2.2, temperature of 70°C and extraction time of 60 min. The three *Aspergillus* species: *A. niger, A. fumigatus* and *A. flavus,* grown in media containing mango peel, produced significant quantities of pectinases. Crude enzymes from *A. niger* and *A. fumigatus* had their highest activities on day 4, while *A. flavus* had its highest activity on day 5 (Figure 1). Figure 2 shows the pectinase activities of mass-produced crude extract. The polygalacturonase activities of *A. niger*, *A. fumigatus* and *A. flavus* obtained from ammonium sulphate precipitation of pectinases were 45.92, 41.69 and 51.19 µmol/min, respectively, at 80% ammonium sulphate saturation (Figure 10). Tables 1, 2 and 3 show the percentage yield and purification fold of pectinases from the three pectinolytic microorganisms after dialysis, while the specific activity of the partially purified enzyme is presented in Figure 3. The effect of pH on the activity of pectinases from the three microorganisms is shown in Figure 4. Figure 4 also shows minor pH peaks for pectinases from *A. fumigatus, A. flavus* and *A. niger*. The effect of temperature on pectinase activities of the three isolates is presented in Figure 5. The effect of temperature

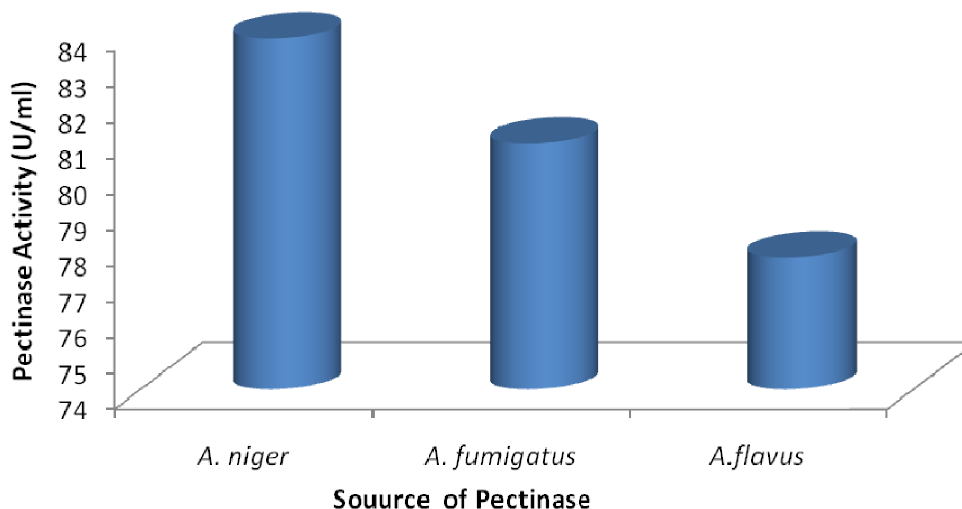

Figure 2. Pectinase activity of crude extracts from the three *Aspergillus* species.

Table 1. Purification of pectinases from *A. niger.*

Purification step	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude enzyme filtrate	500	310	41900	135.16	1	100
Ammonium sulphate precipitation	20	15.6	1131.6	72.54	0.54	2.70
Dialyzed enzyme	23	10.58	2311.04	218.43	1.62	5.40

μmol/min = Unit (U).

Table 2. Purification of pectinases from *A. fumigatus.*

Purification step	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude enzyme filtrate	500	345	40430	117.19	1	100
Ammonium sulphate precipitation	20	21.4	2073.2	96.88	0.83	5.13
Dialyzed enzyme	22.7	14.76	3095.83	209.82	1.79	7.66

μmol/min = Unit (U).

Table 3. Purification of pectinases from *A. flavus.*

Purification step	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude enzyme filtrate	500	300	38840	129.47	1	100
Ammonium sulphate precipitation	20		1203.2	79.16	0.61	3.10
Dialyzed enzyme	22.5	9.65	2326.5	240.56	1.86	5.99

μmol/min = Unit (U).

on the activity of pectinases at minor pH peaks is shown in Figure 6. Table 4 shows pectinase characterisation from different *Aspergillus* species. Figures 7, 8 and 9 show the Lineweaver-Burk plot of initial velocity data at

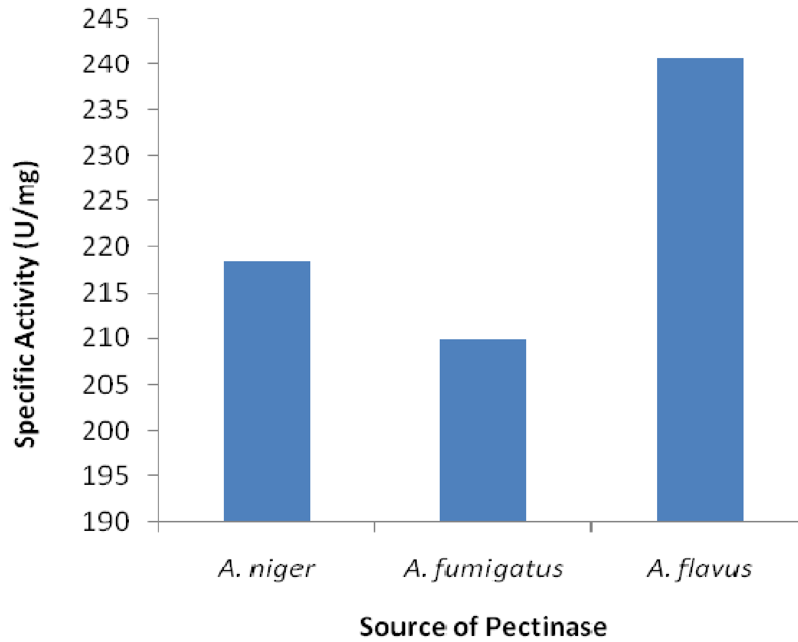

Figure 3. Specific activity of partially purified enzymes from different *Aspergillus* species.

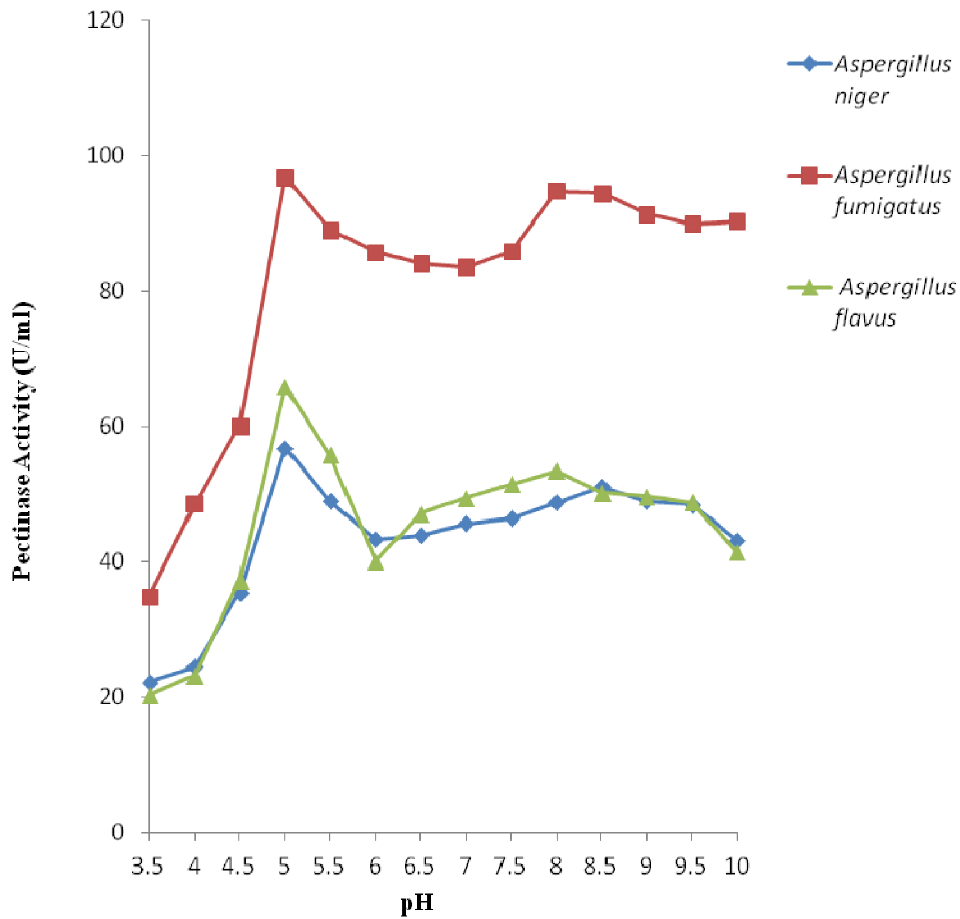

Figure 4. Effect of pH on the activity of pectinases from the three *Aspergillus* species.

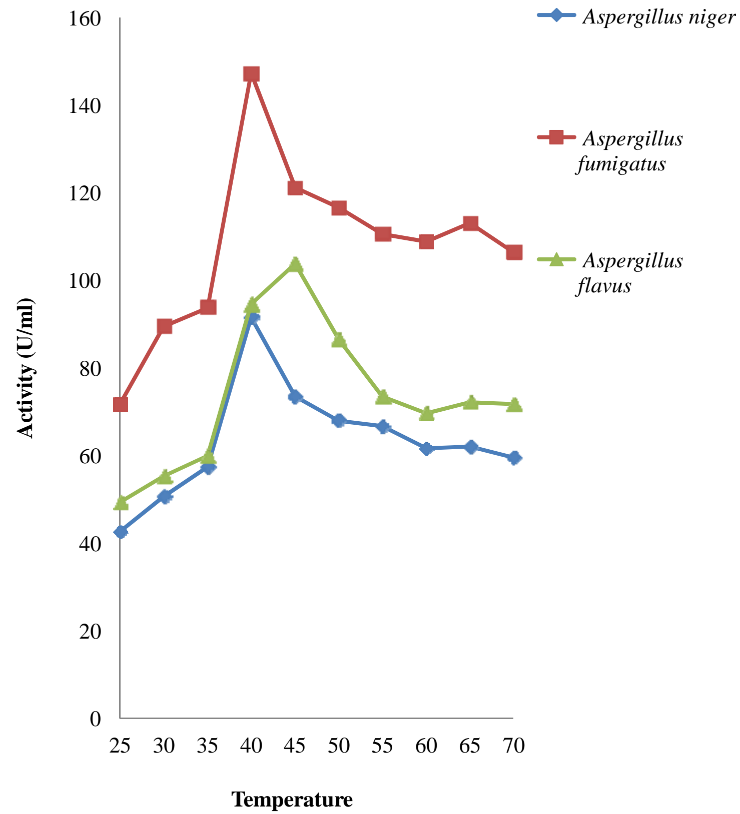

Figure 5. Effect of temperature on the activity of pectinases from the three *Aspergillus* strains.

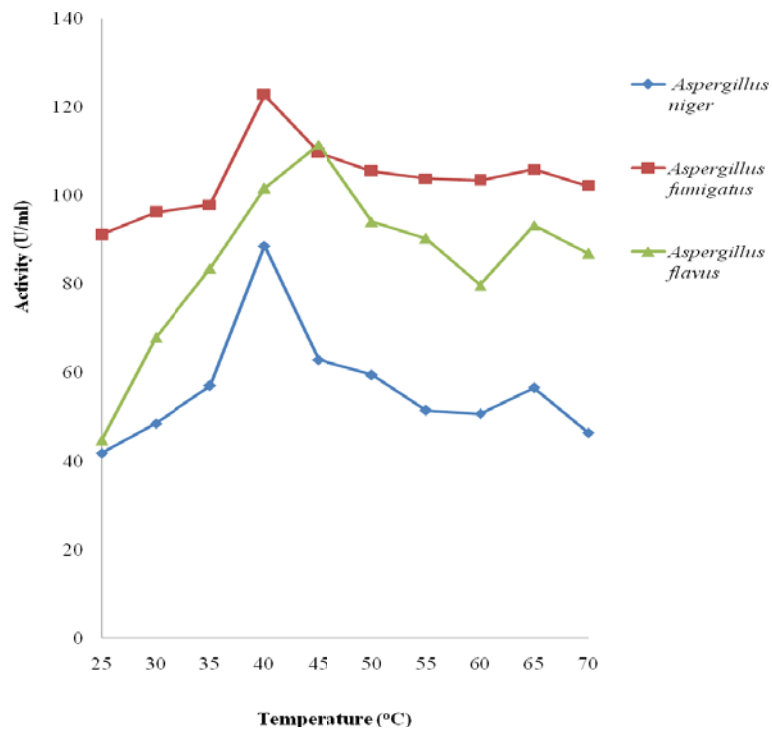

Figure 6. Effect of temperature on the activity of pectinases at minor pH peaks.

Table 4. Pectinase characterization.

Parameter	*A. niger*	*A. fumigatus*	*A. flavus*
pH	5.0	5.0	5.0
Temperature (°C)	40	40	45
V_{max} (µmol/min)	35.34	68.03	60.61
K_m (mg/ml)	0.357	0.156	0.261

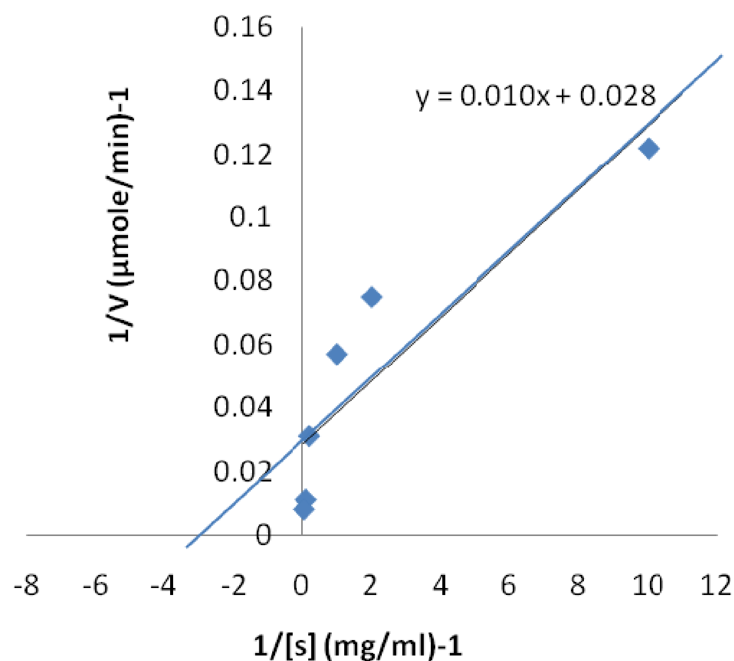

Figure 7. Lineweaver-Burk plot of pectinases from *A. niger*.

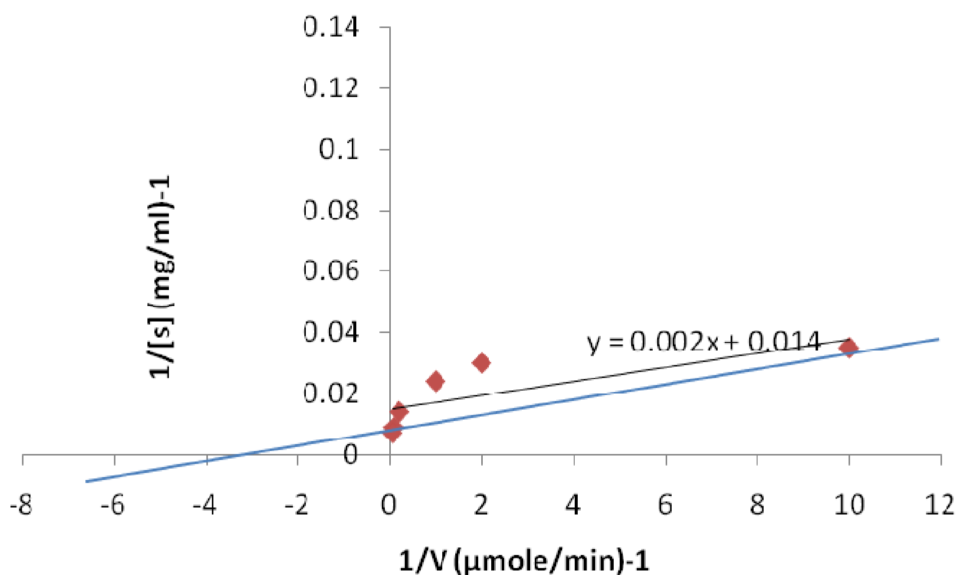

Figure 8. Lineweaver-Burk plot of pectinases from *A. fumigatus*.

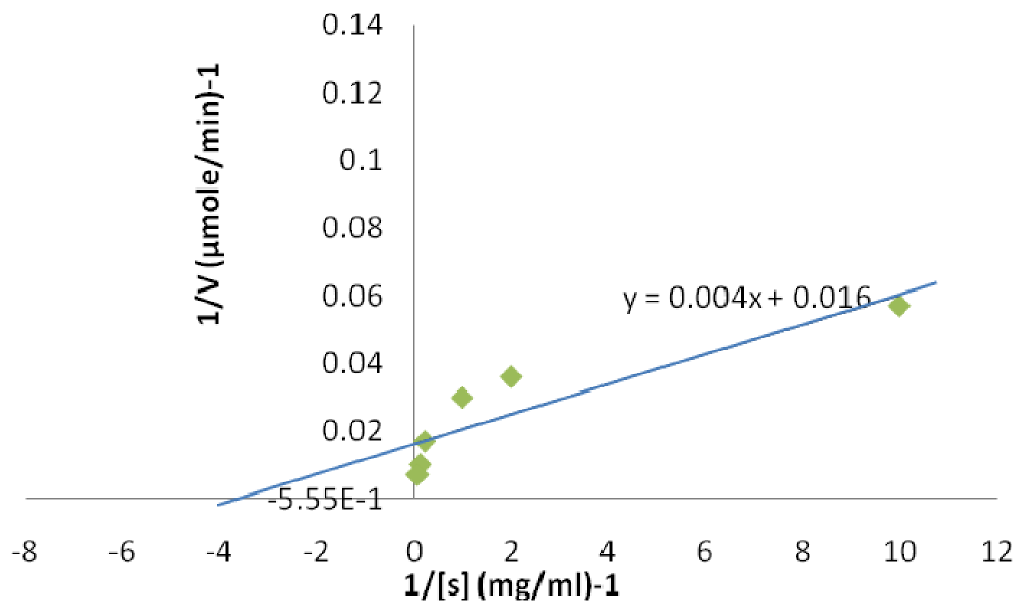

Figure 9. Lineweaver-Burk plot of pectinases from *A. flavus*.

data at different concentrations of mango pectin extract for pectinases from *A. niger, A. fumigatus* and *A. flavus,* respectively.

DISCUSSION

Pectin extraction

The percentage yield is comparable with the results obtained by Sharma et al. (2006), Rouse and Crandall (1976), Hussain et al. (1991) and Rehman et al. (2004). Any differences in the yields may have resulted from the differences in mango species, extraction technique, changes in pH, temperature and extraction time (Kertesz 1951; Rehman et al., 2004).

Pectinase production

The results showed that *A. niger, A. fumigatus* and *A. flavus* can grow well on mango peels. Pectinases production was analyzed over a period of 7 days by studying polygalacturonase (Pg) activities of extracted crude enzymes (Figure 1). Crude enzymes from *A. niger* and *A. fumigatus* had their highest activities (41.5 and 40.22 µmol/min, respectively) on day 4, while *A. flavus* had its highest activity (38.89 µmol/min) on day 5 (Figure 1). The peak days were chosen for optimal pectinase production by individual species. Pectinase activities of crude extract from *A. niger, A. fumigatus* and *A. flavus* were 83.80, 80.86 and 77.68 U/ml, respectively (Figure 2).

Partial purification

The polygalacturonase activities of *A. niger*, *A. fumigatus* and *A. flavus* obtained from ammonium sulphate precipitation of pectinases were 45.92, 41.69 and 51.19 µmol/min, respectively, at 80% ammonium sulphate saturation (Figure 10) which is comparable with the reports of Buga et al. (2010) and Adejuwon and Olutiola (2007) for pectinase from *A. niger* and *Lasidioplodia theobromae,* respectively. The results show that the purification fold was 1.62, 1.79 and 1.86 for *A. niger*, *A. fumigatus* and *A. flavus*, respectively (Tables 1, 2 and 3). The differences in these values might be due to differences in *Aspergillus* strains since pectinase production by filamentous fungi varies according to the strain and other genetic factors (Souza et al., 2003). Total protein decreased from crude values of 310, 345 and 300 mg to dialyzed values of 10.58, 14.76 and 9.65 mg for *A. niger, A. fumigatus* and *A. flavus,* respectively during the purification process (Tables 1, 2 and 3). The reduction in total protein from crude values of 310, 345 and 300 mg to dialyzed values of 10.58, 14.76 and 9.65 mg for *A. niger, A. fumigatus* and *A. flavus,* respectively during purification process might be due to loss of unwanted proteins. In addition, the increase in specific activities of enzymes from the three sources from crude values of 135.16, 117.19 and 129.47 U/mg to values of 218.43, 209.82 and 240.56 U/mg (Tables 1, 2 and 3) for *A. niger*, *A. fumigatus* and *A. flavus,* respectively after dialysis might result from the loss of unwanted proteins that interfere with the enzyme activity, thus leading to an increase in specific activity of the enzyme. However, increment in specific activity is a measure of purification

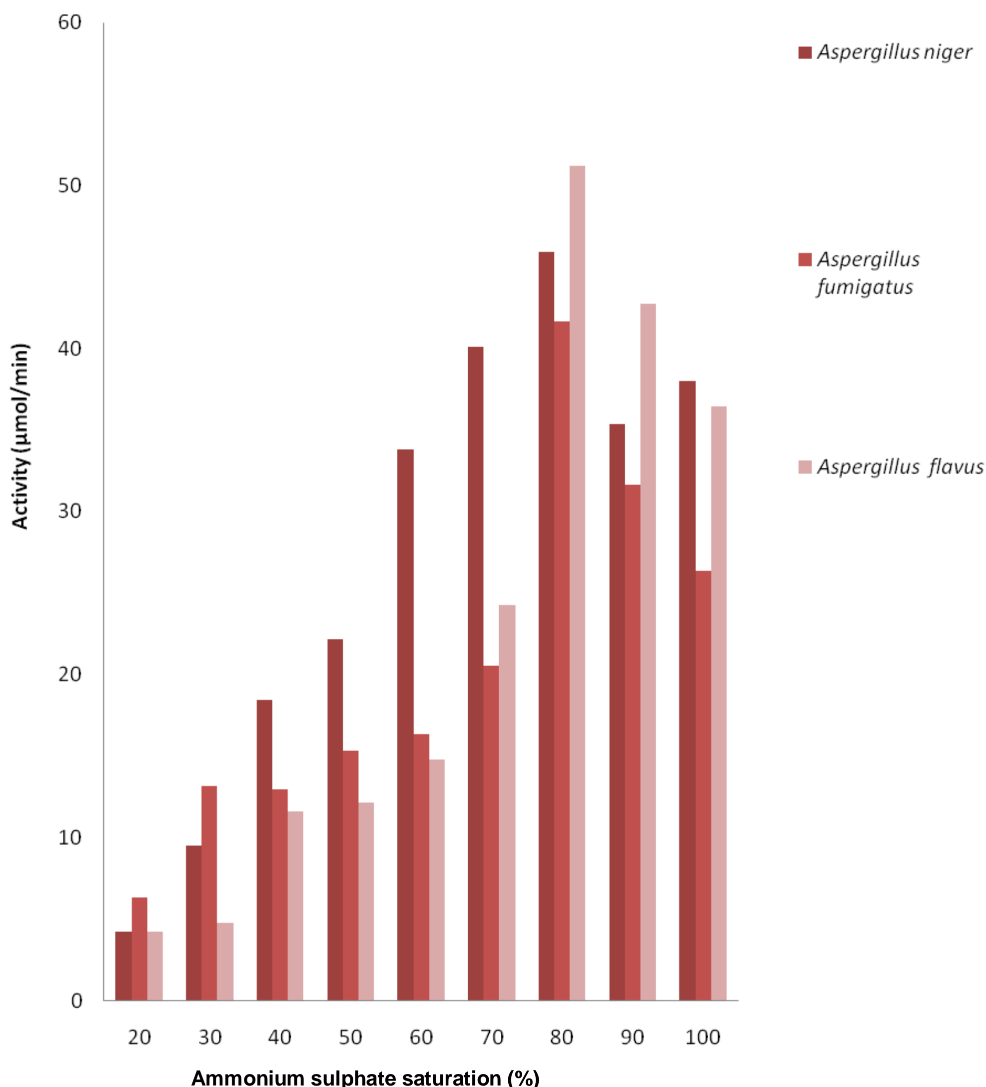

Figure 10. Ammonium sulphate precipitation profile of pectinases from the three *Aspergillus* strains.

achieved, indicating that pectinases from *A. flavus* that have the greatest enzyme specific activity, were the most purified. They were followed by pectinases produced by *A. niger* and then *A. fumigatus,* after partial purification (dialysis).

Pectinase characterization

The optimal pH of 5.0 was obtained for the pectinases from the three *Aspergillus* species (Figure 4) while an optimal temperature of 40°C was obtained for *A. niger* and *A. fumigatus* and 45°C for *A. flavus,* respectively (Figure 5). These results are in consonance with the reports of Favela-Torres et al. (2006), Niture and Pant, (2001) and Manachini et al., (1987). Figure 4 also shows minor peak activities for *A. fumigatus* and *A. flavus* at pH 8.0 and *A. niger* at pH 8.5. At pH of 8.0, pectinases from *A. fumigatus* and *A. flavus* indicated an optimal temperature of 40 and 45°C respectively, while that from *A. niger* indicated a temperature optimum of 40°C at pH of 8.5 as shown in Figure 6. The minor peaks at pH 8.0 for *A. fumigatus* and *A. flavus* and 8.5 for *A. niger* in Figure 4 may represent optimal pH for an isoform of pectinase, possibly pectin lyase. Pectin lyases have pH optima in the alkaline range 7.5-10.0 and temperature optima of 40-50°C (Jayani et al., 2005).

Kinetic studies

Kinetic parameters, V_{max} and $K_{m,}$ of the enzymes were calculated from Lineweaver-Burk plot (Figures 7, 8 and 9). Pectinases from *A. niger, A. fumigatus* and *A. flavus*

had V_{max} and K_m values of 35.34 U and 0.367 mg/ml; 68.03 U and 0.156 mg/ml and 60.61 U and 0.261 mg/ml, respectively (Table 4). Thus, the increasing order of V_{max} for the three organisms is *A. niger>A. flavus>A. fumigatus.* K_m values less than 0.15 and up to 5.0 mg/ml (<0.15 to 5.0 mg/ml) and specific activities 8.8 to 7000 U/mg were reported for some fungal pectinases by Lucie (2000). The K_m value obtained in this study is not only comparable but also in agreement with the report by Rombouts and Pilnik (1980) that the K_m for most fungal pectinases is less than 1 mg/ml.

Conclusion

Most work on pectinase microbial production has been oriented in the direction of using pure pectin extract, especially apple pectin extract, as the sole carbon source, while in this work we used mango peels for pectinase production. Our findings provide alternative and cheaper source of substrates for microbial pectinase production. Thus, mango peel could be an attractive and promising substrate especially in submerged fermentation for the production of pectinases by *Aspergillus* species. Pectinase from *A. fumigatus* with the highest yield during the purification process, indicated highest V_{max} and the lowest K_m (highest catalytic efficiency) value at acidic pH of 5.0 and optimum temperature of 40°C. Therefore, it can be employed in industries for hydrolysis of pectic biomass to utilizable bio-products.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Adejuwon AO, Olutiola PO (2007). Pectin lyase activity in culture medium of *Lasidioplodia theobramae*. J. Plant Sci. 2(3):334-340.

Arogba SS (2002). Quality characteristics of model biscuit containing processed mango (*Mangifera indica*) kernel flour. Int. J. Food Pro. 5:249-260.

Bali R (2003). Isolation of extracellar fungal pectinoytic enzymes and extraction of pectin using Kinnow waste as substrate. MSC thesis. Thaper Institute of Enginerring Technology (Deemed University) Punjab, India. pp. 1-67.

Barnett HL, Hunter BB (1972). Illustrated general of imperfect fungi. 3rd edn. Burgess, Minneapolis, Minnesota, USA. pp. 20-130.

Buga ML, Sani I, Adrew JW (2010). Partially purified polygalacturonase from *Aspergillus niger* (SA6). Afr. J. Biotechnol. 9(52):8944-8954.

Favela-Torres E, Volke-Sepulveda T, Viniegra-Gonzalez G (2006). Hydrolyticide polymerizing pectinases. Food Technol. Biotechnol. 44(2):221-227

Harholt J, Suttangkakul A, Scheller HV (2010). Biosynthesis of pectin. Plant Physiol. 153:384-395.

Hussain T, Baig MQ, Rashid H (1991). Extraction and evaluation of pectin from mango peel. Pak. J. Agric. Res. 12:213-216.

Jarvis MC, Briggs SPH, Knox JP (2003). Intercellular adhesion and cell separation in plants. Plant Cell Environ. 26:977-989.

Jayani RS, Saxena S, Gupta R (2005). Microbial pectinolytic enzymes: a review. Process Biochem. 40(99): 2931-2944.

Kaur M, Singh N, Sandhu KS, Guraya HS (2004). Physiochemical, morphological, thermal and rheolgical properties of starches separated from kernels of some Indian mango cultivars (*Mangifera indica L.*). Food Chem. 85:131-140.

Kertesz ZJ (1951). The pectic substance. Interscience Publishers, New York, 68.

Larrauri JA, Ruperez P, Borroto B, Saura-Calixto F (1996). Mango peels as new tropical fiber: preparation and characterization. Lebensmittel-Wissenchaft und- Technol. 29:729-733.

Lowry OH, Rosenbrough PJ, Fass AL, Randall RJ (1951). Protein measurement with Folin-phenol reagent. J. Biochem. 193:265-275.

Lucie P (2000). Pectinase of *Aspergillus niger*: A molecular and Biochemical Characterization. Thesis, Wageningen University. Netherlands. ISBN 90-5808-203-2 21.

Manachini PL, Fortina MG, Parini C (1987). Purification and properties of an endoplygalacturonase produced by *Rhizopus Stolonifer*. Biotechnol. Lett. 9:219-224.

Martin N, de Souza SR, da Silva R, Gomes E (2004). Pectinase production of fungal strains in solid-state fermentation using agro-industrial bioproduct. Braz. Arch. Biol. Technol. 47(5):813-819.

McCready RM (1970). Methods on food analysis. Academic Press, New York and London, 2nd Edition. p. 365.

Miller GL (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. 31:426-428.

Moharram YG, Moustafa AM (1982). Utilization of mango seed kernel (*Mangifera indica*) as a source of oil. Food Chem. 8:269-276.

Niture SK, Pant A (2001). Purification and biochemical characterization of polygalacturonase 11 produced in semi-solid medium by a strain of *Fusarium moniliforne*. Microbiol. Res. 59:305-314.

Puravankara D, Boghra V, Sharma RS (2000). Effect of antioxidant principles isolated from mango (*Mangifera indica*) seed kernel on oxidative stability of *buffaloghee* (butter-fat). J. Sci. Food Agric. 80:522-526.

Rashmi R, Sddalinga Murthy KR, Sneha G, Shabana S, Syama A, Radhika VS (2008). Partial purification and biochemical characterization of extracellular pectinase from *Aspergillus niger* isolated from groundnut seeds. J. Appl. Biosci. 9(1):378-384.

Ravindran V, Sivakanesan R (1996). The nutritive value of mango seed kernels for starting chicks. J. Sci. Food Agric. 71: 245-250.

Rehman ZU, Salariya AM, Habib F, Shah WA (2004). Utilization of mango peel as source of pectin. J. Chem. Soc. Pak. 26(1):73-76.

Rombouts FM, Pilnik W (1980). Pectic enzymes. In: A.h.Rose, editor. Economic Microbiology, Volume 5, Academic press, London. pp. 227-282.

Rouse AH, Crandall PG (1976). Nitric acid extraction of pectin from citrus peel. Proc. Fla. State Hortic. Soc. 89: 166-168.

Sharma BR, Naresh L, Dhuldhoya NC, Merchant SU, Merchant UC (2006). An overview on pectins. Times Food Process. J. 23(2):44-51.

Souza JVB, Silva ES, Maia MLS, Teixeira MFS (2003). Screening of fungal strains for pectinolytic activity: endopolygalacturonase production by *Peacilomyces clavisporus* 2A.UMIDA.1. Process Biochem. 39:455-458.

Wang G, Michailides TJ, Bostock RM (1997). Improved detection of polygalacturonase activity due to *Mucor piriformis* with a modified dinitrosalicylic acid reagent. Phytopathology 87: 161-163.