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Isolation, Partial Purification and Characterization of Pectinase from Water Melon (*Citrullus lanatus*) Rind

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Abstract: Plant wastes could be a potential source of novel pectinases for use in various industrial applications due to their broad substrate specificity with high stability under extreme conditions. Therefore, the application conditions of a novel pectinase from watermelon *Citrullus lanatus* rind were optimized in this study. The specific activity for the crude enzyme was found to be 76.04U/mg protein. After ammonium sulphate precipitation and gel filtration, the specific activities were found to be 77U/mg and 179.18U/mg, respectively. 30% ammonium sulphate saturation was found suitable to precipitate protein with highest pectinase activity. The enzyme exhibited highest activity of 71.35µmol/min at pH 9.5. The optimum temperature for the enzyme was found to be 55°C with activity 111.89µmol/min. The maximum velocity, V_{max} and Michealis constant, K_m determined from Lineweaver-Burk plots of initial velocity data at different concentrations of the pectin were found to be 333.3µmol/min and 35.6mg/ml respectively.

Key words: Pectinases · Watermelon rind · Optimum temperature · Optimum pH and kinetic parameters

INTRODUCTION

Over the years, pectinases have been used in several conventional industrial processes. They are of great significance, with a wide range of applications in the fruit, beverage and textile processing industries, in the treatment of pectin wastewaters, degumming of plant fibers, pulp and papermaking and for coffee and tea fermentation [1, 2]. Pectin substances are the complex polysaccharides present in the middle lamella of plants and are degraded by a group of enzymes called pectinases. Pectinases are classified into Pectin Methyl Esterases (PME) or pectinesterases, Polymethylgalacturonases (PMG), Polygalacturonases (PG), PectateLyases (PGL) and Pectin Lyases (PL) [3] and [4]. Pectinases could be sourced from plant and microorganisms [5] and [6]. Most industrial production of pectinases is limited to some species of bacteria, yeast and fungi. Due to the extensive applications of the enzyme in various industries there is a need to find novel sources of the enzymes due to their current limited production. Furthermore, utilization of agro-industrial residues (in the form of peels and rinds) for enzyme production minimizes environmental pollution and allows the production of high value-added products using an economical process.

Watermelon (*Citrullus lanatus*) is a warm-season crop related to squash, cucumber, pumpkin and is among the important commercial tropical fruits in the World. Watermelon is now used in juice production. Excessive consumption of watermelon generates a lot of wastes in the form rinds. There are different types of enzymes in watermelon rind and thus could be used as a rich and cost effective source for the commercial production of natural and valuable kinds of the enzymes.

The current study is aimed at production of pectinase from watermelon rind and optimization of the pectinase activity by determining the optimum pH, temperature as well as the kinetic parameters (K_M and V_{max}).

MATERIALS AND METHODS

Collection of Plant Material: Watermelon (*Citrullus lanatus*) fruits were purchased from a local market, Ikpa market in Nsukka Local Government, Enugu state, Nigeria.

Methods

Extraction of Crude Pectinase from Water Melon Rind: A quantity of six *Citullus lanatus* fruits were cut into ten large pieces with a stainless knife after washing with distilled water. The rind was then separated from the pulp.

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A further dicing of the rind into smaller pieces of average size (2cm x 1.5cm x 0.5cm) was done to enhance accurate and easy weighing. 200g of *Citullus lanatus* rind was homogenized using wooden mortar and pestle. The enzyme was extracted using 500ml of 0.05M Sodium acetate buffer pH 5.0. This was followed by filtration using cheese cloth.The filtrate was used as the crude enzyme and was stored at 4°C.

Protein Determination: Protein content was determined by the method of *Lowry et al*. [7] using bovine serum albumin as protein standard as contained in Udenwobele *et al.* [8].

Pectinase Activity Assay: Pectinase activity was evaluated by assaying for polygalacturonase (PG) activity of the enzyme. This was achieved by measuring the release of reducing groups from pectin using a modification of the 3, 5-dinitrosalicylic acid (DNS) reagent assay method described by Miller [9] as contained in Wang *et al*. [10] with the following modifications. The reaction mixture containing 0.5ml of 0.5% pectin in 0.05M sodium acetate buffer pH 5.0 and 0.5ml of enzyme solution was incubated for 1 hour. 1ml of DNS reagent was added and the reaction was stopped by boiling the mixture in a boiling water bath for 10min. The reddish brown coloration was stabilized by adding 1ml of Rochelles salt solution followed by 1ml of distilled water. The reaction mixture was allowed to cool and then the absorbance was read using a JENWAY 6405 UV/VIS spectrophotometer (Beckman/Instruments, Inc., Huston Texas) at 575nm. One unit of enzyme activity (U) was defined as the amount of enzyme that catalyzes the release of one micromole of galacturonic acid per minute.

Partial Purification of the Pectinase

Ammonium Sulphate ($(NH_3)_2SO_4$) Precipitation: The crude enzyme preparation was made up to 30% $(NH_4)_2SO_4$ saturation with solid $(NH_4)_2SO_4$. This was kept at 4°C for 30hrs, thereafter it was centrifuged using Cole-palmer VS-13000 microcentrifuge at 4000 rmp for 30min. The precipitate was collected and redisolved in 0.05M acetate buffer pH 5.0. The pectinase activity and protein were determined as described above.

Gel Filtration: A volume 30ml of the precipitated protein was introduced into a (50 x 2.5cm) gel chromatographic column and subjected to gel filtration using sephadex G-25. The gel was pre equilibrated with 0.05M sodium acetate buffer pH 5.0. Fractions were collected at a flow rate of 5ml/9min. The protein concentration of each fraction was monitored using a JENWAY 6405 UV/VIS spectrophotometer (Beckman/Instruments, Inc., Huston Texas) at 280nm. The pectinase activity of each fraction was assayed as earlier described and the active fractions were pooled and stored at -10°C.

Enzyme Characterization

Effect of Ph on Pectinase Activity: The pectinase activity was determined in a pH range of 3.5-5.5 in 0.05M sodium acetate buffer, pH 6.0-7.5 0.05M sodium phosphate buffer and 8.0-9.0 0.05MTris-HCl buffer at interval of 0.5. The pectinase activity was assayed as described above using pectin as substrate.

Effect of Temperature on Pectinase Activity: The effect of temperature on pectinase activity was determined by varying the temperature from 35 to 70°C at interval of 5°C. The pectinase activity was assayed as described above using pectin as substrate using 1% pectin as the substrate.

Effect of Substrate Concentration on Pectinase Activity: The effect of substrate concentration on pectinase activity was determined by incubating the enzyme with 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50mg/ml of pectin at pH 5.5 and 45°C.The maximum velocity V_{max} and Michaelis constant K_m were determined from the double reciprocal plot of initial velocity data at different concentrations of pectin.

RESULTS AND DISCUSSION

A volume 1litre of the crude enzyme was isolated from 200g of water melon rind and the protein concentration was found to be 0.447mg/ml. The increase in protein to 0.714mg/ml after ammonium sulphate precipitation could be due to precipitation of every protein in the extract while the decrease in protein concentration to 0.126mg/ml after gel filtration could be due to desalting and separation of the proteins based on their molecular sizes. The specific activity for the crude enzyme was found to be 76.04U/mg protein. After ammonium sulphate precipitation and gel filtration, the specific activities were found to be 77U/mg and 179.18U/mg, respectively. This suggests that as purification steps increases, the enzyme activity also increases Table 1.

Table 1: Purification table for pectinase isolated from water melon rind

Enzyme	Volume (ml)	Protein (mg/ml)	Total Protein	Activity (µmol/min)	Total Activity (µmol/min)	Specific Activity U/mg	Purification Fold	% Yield
Crude	1000	0.447	447.00	33.99	3399.00	76.04	1.00	100
$(NH_4)_2SO_4$	700	0.714	499.80	54.62	38234.00	77.00	1.01	14.7
Gel filtration	100	0.126	12.60	22.58	225.80	179.18	2.33	2.52

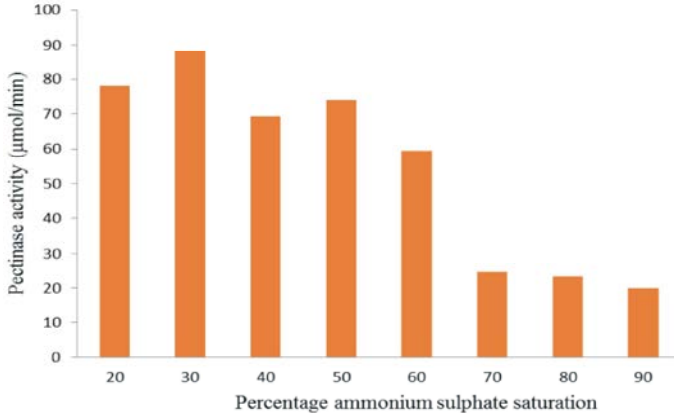

Fig. 1: Ammonium sulphate precipitation profile

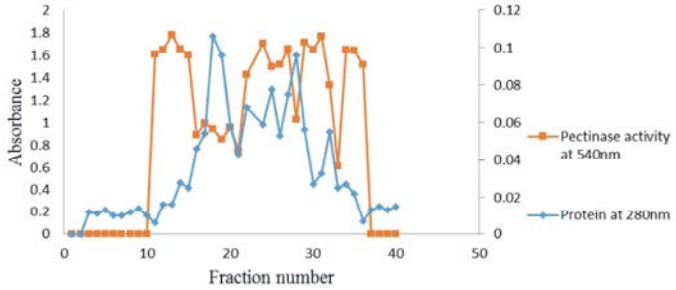

Fig. 2: Elution profile for pectinase isolated from water melon peels

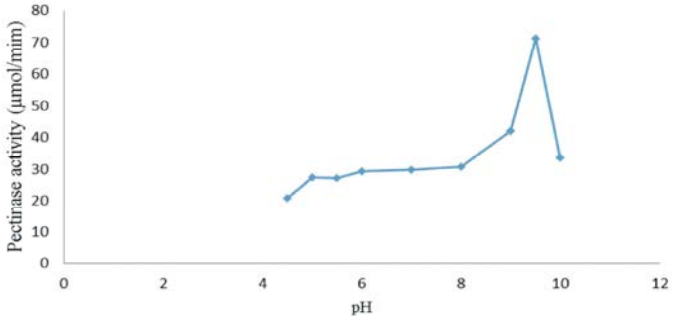

Fig. 3: Effect of pH on pectinase activity

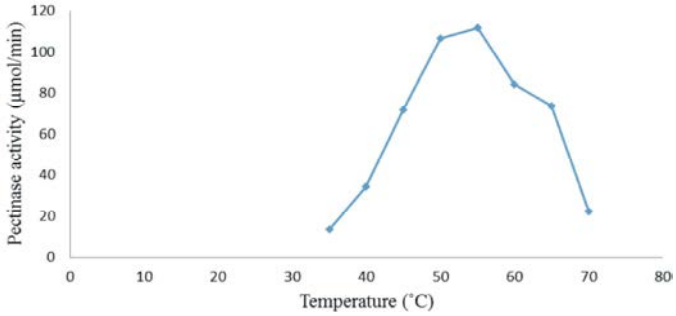

Fig. 4: Effect of temperature on pectinase activity

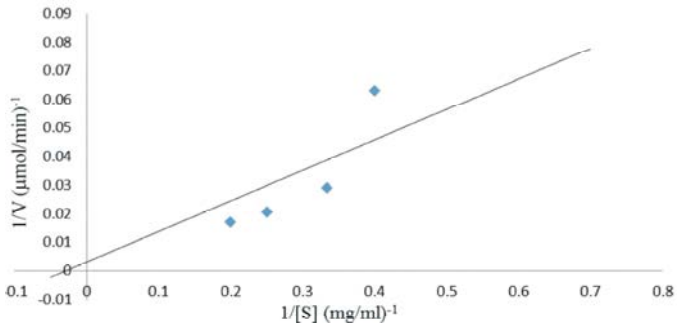

Fig. 5: Linewaever-Burk plot of initial velocity data at different pectin concentration

The studies show that 30% ammonium sulphate saturation was found suitable to precipitate protein with highest pectinase activity (Figure 1). From the elution profile during chromatography on sephadex G-25 (Figure 2), the fractions with highest activity were pooled and assayed for pectinase activity.

Figure 3 shows that increase in pH was accompanied by an increase in enzyme activity up to pH 9.5 beyond which, the enzyme activity decreased steadily making 9.5 the optimum pH for pectinase activity The enzyme exhibited highest activity of 71.35µmol/min at pH 9.5. These results suggest that the enzyme do very well at alkaline pH 9.5 and could be applied industrially at this pH. Nor and Mehrnoush [1] reported optimum pH of 8 using pectinase extracted from red pitaya (*Hylocereuspolyrhizus*) peel.

Figure 4 shows that increase in temperature from 35°C to 55°C was accompanied by increase in pectinase activity beyond which the enzyme activity decreases making 55°C the optimum temperature with activity 111.89µmol/min. The increase in the enzyme activity as temperature increases may be as a result of change in the enzyme conformation which brings the essential residues to close proximity for catalysis. The decrease in the activity could be as a result of thermal denaturation at high temperature. Similar results were also reported for polygalacturonase by *Aspergillus awamori* and *Aspergillus niger* [11]. Exo-polygalacturonase from *Monascus* and *Aspergillus sp*. Freitas *et al.*[6] exhibited maximum activity at 60 and 50°C, respectively. Udenwobele *et al.* [8] reported optimum temperature of 40°C and 45°C using *Aspergillus niger, Aspergillus fumigatus and Aspergillus flavus*, respectively. The maximum velocity, V_{max} and Michealis constant, K_m determined from Lineweaver-Burk plots of initial velocity data at different concentrations of the pectin were found to be 333.3µmol/min and 35.6mg/ml, respectively. Baladhandayutham and Thangavelu [5] reported Km and Vmax to be 79.3 and 1.724 hr.ml/U respectively for pectinase from *Aspergillus awamori*. V_{max} and K_m show that the enzyme has higher affinity for pectin used in this work.

CONCLUSION

The results of the study suggest that the natural and valuable enzyme from watermelon rind with its unique characteristics such as high stability at alkaline pH and temperature 55°C can be used as a potential enzyme in different types of industries and biotechnological applications.

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