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PURIFICATION AND PARTIAL CHARACTERIZATION OF PROTEASES FROM PEELS OF COCOYAM (COLOCASIA ESCULENTA)

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ABSTRACT

Proteases were isolated from peels of cocoyam using ammonium sulphate fractionation and purified using gel filtration on sephadex G – 150. The isolated enzymes were assayed and activity measured using casein as substrate. The enzymes activity was found to be optimum at pH 7.0 and at a temperature of 37.5°C. The km was calculated to be 5×10^{-5} mM and the Vmax was 0.714mol/min/ml. The purification of the enzymes on sephadex G – 150 gave four peaks, with peak I showing protease activity the most. The molecular weight of each peak was estimated by gel filtration technique on sephadex G – 75 to be aproximately 18, 21, 24 and 32 Kda.

KEY WORDS: Proteases, Cocoyam, Peels.

INTRODUCTION

In recent years, proteases have been isolated and purified from various biological sources of animals, plant and microorganisms(1). The presence of protease in legumes, soy beans and potato tubers has been indicated (2). The primary functions of this enzymes is to degrade proteins. To prevent protein degradation these enzymes are synthesized in their inactive forms called zymogens(3). This is a protective mechanism preventing the digestion of tissues, which produce them. Secretion in the form of zymogen is a characteristic feature of all extracellular proteases of animal origins(3). Plant and microbial proteases of intracellular origin are synthesized in their active forms(4). Proteases have been used for a variety of purposes of economic importance, plant and animal proteases are used in chill proofing of beer, tenderlization of meat, cheese production and to generate peptide fragments for the elucidation of the primary structure of proteins(5). The purpose of this work is to find possible use for peels of Cocoyam (colocasia esculenta) instead of polluting our environment with it. Therefore, this investigation was carried out to isolate, partially purify and characterise proteases from cocoyam peels.

MATERIALS AND METHODS

Cocoyam Tubers: Cocoyam tubers were purchased from a local market in Ojo Local Government area of Lagos State, Lagos Nigeria.

ISOLATION

Cocoyam tubers were washed properly using distilled water and peeled. 10grams of peels were blended with 90ml of 0.2M phosphate buffer (pH. 7.2) using a warring blender. The blended mixture was filtered using clean white piece of cloth. The filterate was centrifuged using refrigerated centrifuge at 5000 rpm for 30mins. The filtrate was carefully collected and subjected to ammoinum sulphate fractionation at 30%, 50% and 70% saturation levels. The precipitate in each case was collected by centrifugation at 4000 rpm for 15 min. The pellet was dissolved in small amount of buffer solution and dialysed against distilled water for 48 hours.

Protein Determination:- Protein determination of the crude as well as the purified enzymes was carried out using Biuret method,(6).

ENZYME ASSAY

Enzyme assay was performed using Kunitz Caseionolytic assay (7). 1.0ml of the enzyme source was added to 3.0ml of 0.5% casein. The reaction mixture was incubated for 30mins at 37°C and the reaction terminated by adding 5ml of 5% TCA. The solution was kept for 30mins at room temperature and then centrifuged to measure the absorbance of

the supernatant at 280nm. One unit of enzyme was defined as an increase of 0.1 in absorbance of TCA soluble casein hydrolysis product at 280nm.

PURIFICATION PROCEDURE

The dialysed sample of the 50% ammonium sulphate saturation fraction which shows enzymes activity the most was carefully layered on the pre-swollen sephadex G-150 which was packed into a pharmacia chromatrographic column (1x24cm). The column was previously equilibrated with 0.1M phosphate buffer (pH 7.2) and the protein was also eluted using the same buffer. A flow rate of 0.3ml/min was maintained. The absorbency of the fractions was measured at 280nm.

CHARACTERIZATION

Determination of Km and Vmax

The rate of enzymes catalyzed reaction was determined using the Michealis-

Menten equation $Vo = \frac{Vmax(s)}{Km+(s)}$. this was done

using Kunitz caseinolytic assay(7) but with a range of substrate concentration from 0.2ml to 2.0ml.

EFFECT OF TEMPERATURE:

The effect of temperature on the enzyme activity was done at a temperature range of between 15°C and 55°C with an interval of 5°C. Kunitz caseinolytic assay method was used(7).

EFFECT OF PH

This was carried out by the method described above but now varying the pH of reaction mixture between a range of 2-11 with an interval of 1.0 using 0.1M Hcl and 0.1M NaOH.

Molecular Weight Determination

The molecular weight of fraction from each peak showing absorbance at 280nm were pooled individually, concentrated by freeze drying and their molecular weight determined by gel-filtration using sephadex G – 75 as described by Okotore and Aboderin(8). Standard proteins used to calibrate the column include, Bovine Serum albumin, chymotrypsin, lysozyme and myoglobin. The partition coefficients were determined from the equation.

$$Kav = (Ve-Vo)/(Vt-Vo).$$

Where Ve is the elution volume ot the protein, Vo is the void volume of the column and Vt the total bed volume.

Absorbance was taken at 280nm. The molecular weight of the proteins was obtain differently by Extrapolation on the graphical plot of partition co-efficient Kav, against log molecular weight of the standard proteins.

Results.

PROTEIN DETERMINATION

The result of protein determination shows that the crude extract contain 0.71mg/ml protein while the 50% $(NH_4)_2SO_4$ Fraction contain more of the protein (table 1).

ENZYME ASSAY

The 50% $(NH_4)_2SO_4$ Fraction was found to contain the most active fraction of the enzymes with specific activity of 15.75×10^{-1} unit/mg protein while the 30% and 70% fractions have specific activity of 9.33×10^{-1} and 6.53×10^{-1} unit/mg protein respectively (table 1)

PURIFICATION

The gel filtration of fraction II (50%$(NH_4)_2SO_4$ fraction) which contains the bulk of the protein and whose activity is the highest on sephadex G-150 gave four peaks (fig. 1). Peak I shows protease activity the most with a specific activity of 29.42×10^{-1} unit/mg protein (table 1).

EFFECT OF PH

The enzyme was found to have optimum pH of 7.0 (fig 2)

EFFECT OF TEMPERATURE

Proteases from peels of cocoyam were found to function best at temperature of 37.5°C (fig 3).

DETERMINATION KM AND VMAX

The effect of substrate saturation on the enzyme was determined. The values of Km and Vmax were determined by plotting the graph of I/V vs I/(s) and was found to be 5 x 10^{-5} mM and 0.714 mol/min/ml respectively (fig. 4).

MOLECULAR WEIGHT CHARACTERIZATION

The elution volume (Ve) of the standard proteins were obtain by gel filtration and the calibration curve plotted (fig. 5). The elution volume of the protein in each peak was also determined and the molecular weight was estimated by extrapolation on the standard curve to be Peak I (32Kda), peak II (24Kda), peak III (21Kda), and peak IV (18Kda).

Figure 1: Gel Filtration of II (30-50% $(NH_4)_2$ So_4 Saturation) on Sephadex G-150

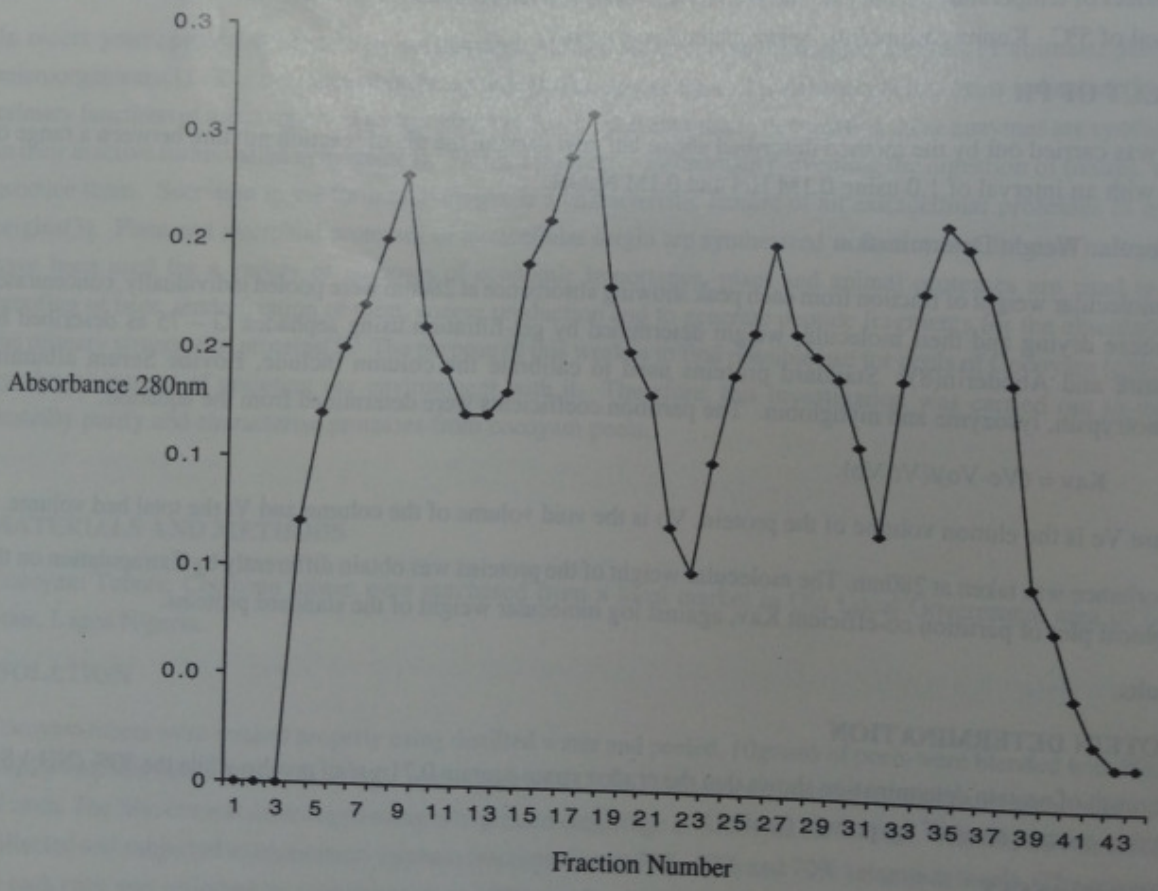


Figure 2: Effect of pH on Protease Activity

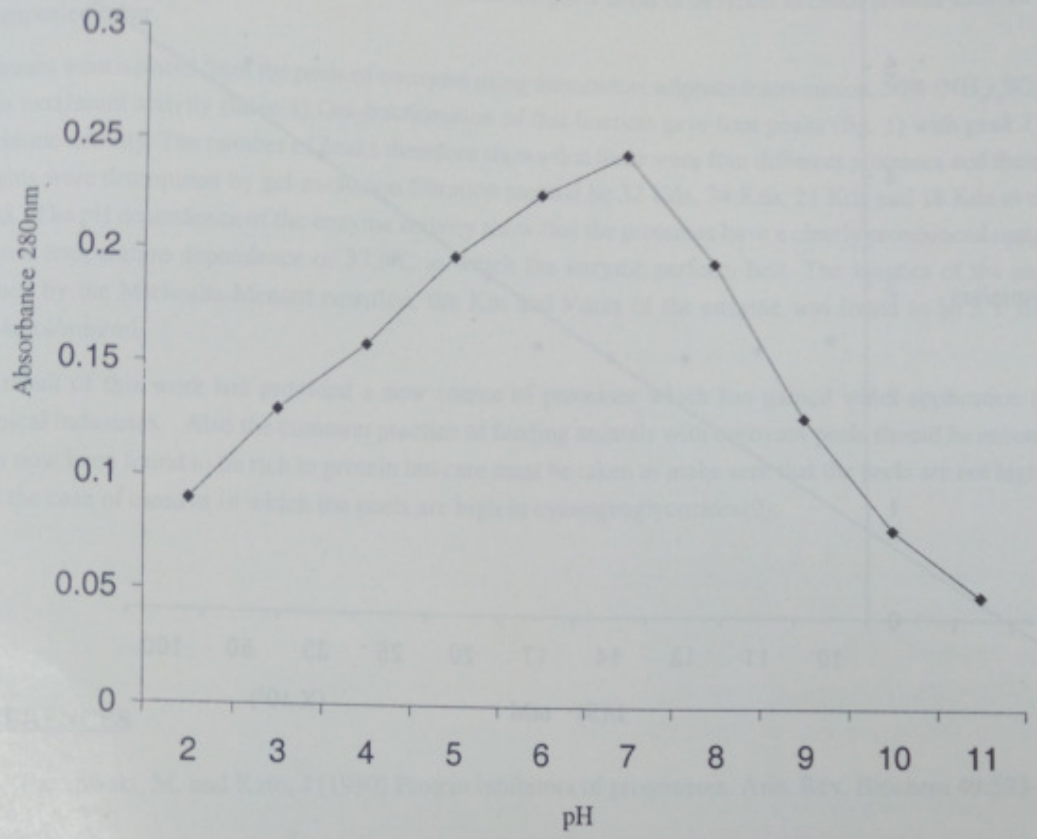


Figure 3: Effect of Temperature of Protease Activity

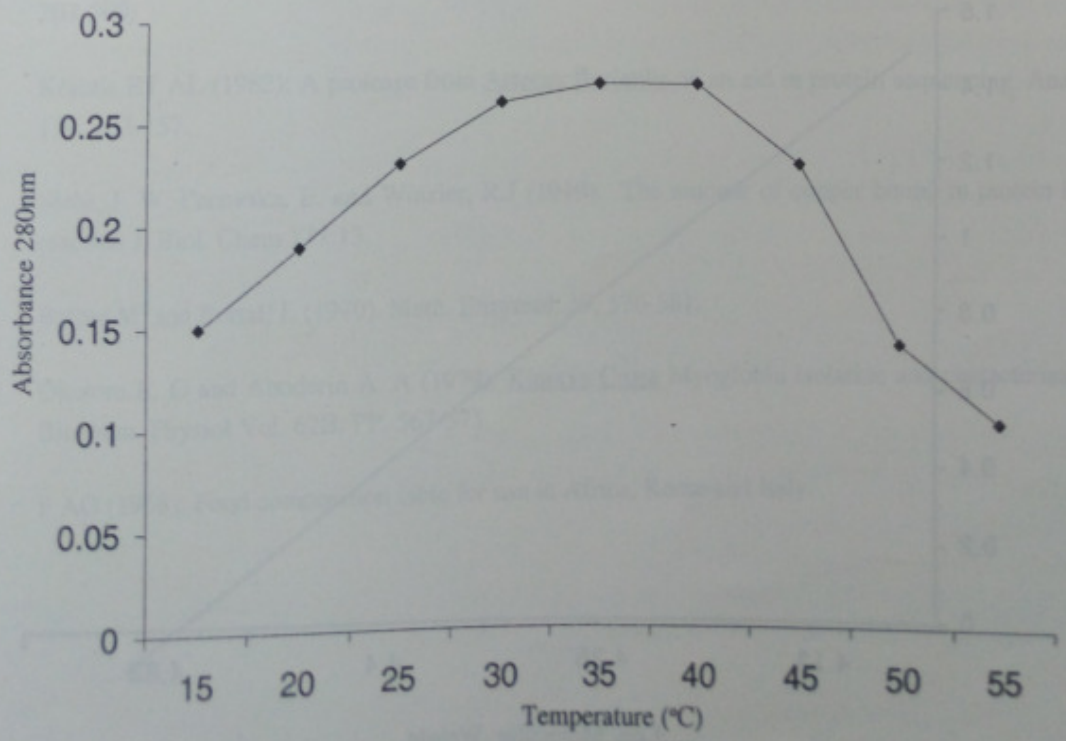


Figure 4: Showing the Kinetics of the Enzyme

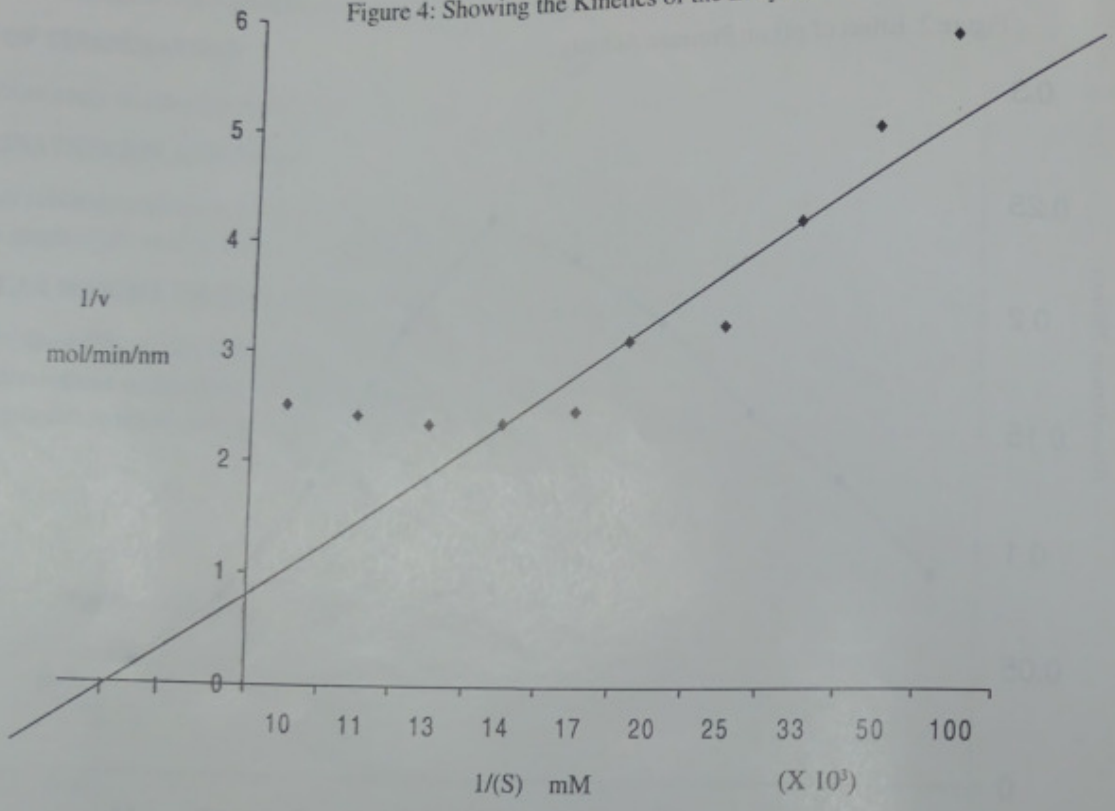
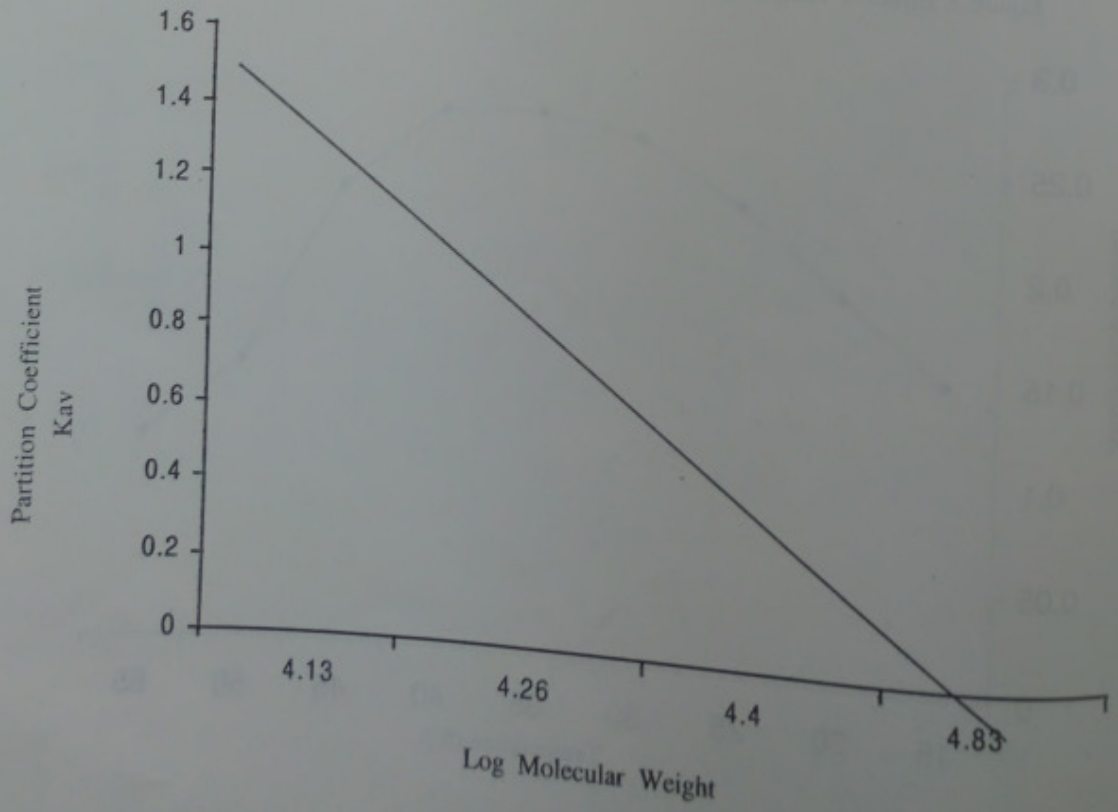


Figure 5: Standard Calibration Curve for the Molecular weight Determination of Proteases from peels of cocoyam by Gel filtration On Sephadex G - 75.



DISCUSSION AND CONCLUSION

This work has demonstrated the presence of proteases in the peels of cocoyam. Similar work has been carried out on sweet potatoes generally (2) and it was found out that the peels tends to be richer in crude protein than the peeled and the unpeeled tuber.

Proteases were isolated from the peels of cocoyam using ammonium sulphate fractionation, 50% $(NH_4)_2SO_4$ saturation gives maximum activity (table 1).Gel-fractionation of this fraction gave four peaks (fig. 1) with peak I having the maximum activity. The number of peaks therefore shows that there were four different proteases and their molecular weights were determined by gel-exclusion filtration method be 32 Kda, 24 Kda, 21 Kda and 18 Kda in order of the peaks. The pH dependence of the enzyme activity show that the proteases have a clearly pronounced optimum pH of 7.0 and temperature dependence of 37.5°C at which the enzyme perform best. The kinetics of the enzyme were defined by the Michealis-Menten equation, the Km and Vmax of the enzyme was found to be 5×10^{-5} mM and 0.714mol/min/ml.

The result of this work has provided a new source of proteases which has gained wider application in food and chemical industries. Also the common practice of feeding animals with cocoyam peels should be encouraged since it has now been found to be rich in protein but care must be taken to make sure that the peels are not high in toxicant as in the case of cassava in which the peels are high in cyanogenglycosides(9).

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