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Original Research Article

Partial Purification and Kinetic Properties of Polygalacturonase from *Chrysophyllum albidum* G. Don Fruit

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ABSTRACT

Polygalacturonase is the major pectic enzyme responsible for hydrolysing pectic substances into their monomeric units. This study evaluated the kinetic properties of extracted and partially purified polygalacturonase from *Chrysophyllum albidum* fruit. Polygalacturonase was extracted from *Chrysophyllum albidum* fruit and partially purified using ammonium sulphate precipitation (80% saturation), dialysis, and gel filtration (Sephadex-G 100). Protein content and polygalacturonase activity were assayed, and the effects of pH, temperature, and substrate concentration on the enzyme activity were determined. The protein concentration and polygalacturonase activity for the ripe fruit were 1.35 mg/mL and 76.35 U/mg protein, respectively, while for the unripe fruit, it was 0.925 mg/mL and 1.59 U/mg protein. Upon partial purification, five fractions (fraction 18, 21-24) had the highest polygalacturonase activity. The optimum pH and temperature for *Chrysophyllum albidum* juice extract were 4.5 and 40°C, respectively. The enzyme activity increased with an increase in substrate concentration. The V_{max} for polygalacturonase was 4.42 U/mg protein, and K_m was 1.38 mg/mL. In conclusion, *Chrysophyllum albidum* fruit is a source of polygalacturonase which could be explored.

***Keywords*:** Pectinase, Polygalacturonase, *Chrysophyllum albidum*, Partial purification, Enzyme activity

Introduction

Pectinases constitute a unique group of enzymes that catalyse the degradation of pectic polymers' in the plant cell wall.[1] The major polysaccharides found in the plant cell wall are cellulose, hemicelluloses and pectin. They are complexed such that the cellulose-hemicellulose network is entrenched in the pectin matrix to give rigidity to the cell wall.[2] Polygalacturonase is the major pectic enzyme responsible for hydrolysing pectic substances into their monomeric units. It is involved in degrading the middle lamella and cell walls by catalysing α-(1–4) galacturonan linkages hydrolytic cleavage.[3,4] This action also makes it an important enzyme involved in pectin structure change that accompanies fruit ripening. Pectinases have industrial uses, particularly in the preparation of fruit juices and vegetable juices where it is used to increase the juice yield and improve the extraction of colourings and aromas.[5-7] They can also be used to treat wastewater in the textile and paper industries.[8] Polygalacturonases play a very important role in the fruit ripening process, winemaking, clarification of fruit juice, textural softening of fruits.[9] *Chrysophyllum albidum* is a perennial tree that grows up to 40 m high, stems with buttress root and girth varying from 1.5 to 2 m in both primary and secondary forests. The fruits are consumed widely in Nigeria and the entire West Africa sub-region.[10] It is locally known as 'Agbalumo' in South-Western Nigeria, 'Agwaluma' among the Hausas, 'Udala' or 'Udara' in South-Eastern Nigeria, 'Olien' in Benin, and 'Alasa' in Ghana. The fruit is a large berry whose pulp is rich in iron, vitamin C and saturated fatty acids.[11]

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Over the years, pectinases have been produced primarily through fermentation with fungal cultures of *Aspergillus, Penicillium, Bacillus* and *Trichoderma* species and other sources such as bacteria and yeasts.[12-16] Plant pectinases have high activity and stability in a wide range of temperature and pH.[17] These properties have intensified interest in seeking plant sources of pectinase with good properties for industrial use, which could lead to a more efficient and cheaper yield of fruit juice. Therefore, the present study seeks to characterize and determine the kinetic properties of polygalacturonase from the fruit extract of *Chrysophyllum albidum.*

Materials and Methods

Chemicals and Reagents
All chemicals and reagents of analytical grade were obtained from Sigma-Aldrich Chemie, Klincent and Qualikems Laboratory.

Plant Collection
Ripe and unripe fruits of *Chrysophyllum albidum* were sourced in January 2014 from Agbara market, Ota, Ogun State, Nigeria. The fruits were identified by Dr. J. O. Popoola from Biological Sciences Department of Covenant University, Ota and deposited in the herbarium (CA/CUBio/G177). Physical properties of the fruits such as texture, colour, and so on were thereafter examined.

Protein and Polygalacturonase Activity Assay
The protein content of the enzyme solution was estimated by the method of Lowry, Rosebrough[18] with bovine serum albumin (BSA) as standard. Polygalacturonase activity was assayed according to the method of Miller[19] using galacturonic acid as standard.

Partial Purification of Polygalacturonase
The partial purification of polygalacturonase in the crude extracellular enzyme was carried out by ammonium sulphate precipitation and gel chromatography. 100 mL of the normal crude extract was dissolved in 20 mL acetate buffer (0.05 M, pH 4.0) in a 250 mL beaker in which 56.1 g of ammonium sulphate was dissolved to 80% saturation. The

crude enzyme-ammonium sulphate solution was centrifuged at 6000 rpm for 10 min. The supernatant was carefully decanted, and the residue was dissolved in 20 mL of acetate buffer (0.5 M, pH 4.0) and left overnight at 4°C. Molecular sieving was done by using Sephadex G-100 (Sigma). Three grams of Sephadex G-100 was suspended in 60 mL distilled water and packed into a glass column (1×50 cm) at room temperature. Acetate buffer (0.1 M; pH 4.0) was run through the loaded gel to wash the gel, after that, 5 mL of the dialysed enzyme was poured on top of the Sephadex-packed column. Acetate buffer (0.1 M; pH 4.0) was passed through the column at a constant flow rate of 9 drops per minute. Fractions of 5 mL volume were each collected and assayed for their protein concentration and enzyme activity at 280 nm and 540 nm, respectively. Fractions found to have high polygalacturonase activity were pooled together and concentrated by freeze-drying.[20]

Kinetic Properties of Polygalacturonase
The kinetic properties of polygalacturonase using different parameters were carried out according to the method described by Chinedu.[20]

pH: The effect of pH on polygalacturonase activity was determined by incubating the enzyme at pH values ranging from 3.0 to 9.0. These substrates were prepared in two buffer solutions: 0.1 M sodium acetate buffer (pH 3.0 to 6.0) and 0.1 M phosphate buffer (pH 6.5 to 9.0).

Temperature: The effect of reaction temperature on polygalacturonase activity was determined by incubating the enzyme at varying temperatures from 30 to 100°C for 30 min. The polygalacturonase activity for the different temperatures was determined.

Time course: The time course of the enzyme was determined by measuring the enzyme activity at different periods of incubation (10 - 70 min) under standard assay conditions of pH 4.0 and 50°C. The polygalacturonase activity at different time intervals was determined. The activities were plotted against their corresponding incubation times.

Effect of substrate concentration: The effect of various substrate concentrations (0.1 to 1.0 mg/mL) on the enzyme activity was studied under the standard condition for the enzyme (pH 4.0 and 50°C) for 30 min. The enzymes Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) were obtained using the Line-Weaver Burk plot.

Statistical Analysis
All the assays were conducted in triplicate, and the result was expressed as mean ± standard error of mean using Microsoft Excel 2016.

Results and Discussion

The physical characteristics of *Chrysophyllum albidum* fruits (ripe and unripe) were summarised in Table 1. They were both oval-shaped with semi oval-shaped seeds. Nonetheless, the exocarp and mesocarp colour was green and orange for the unripe and ripe fruit, respectively. Protein concentration and polygalacturonase activity for ripe *Chrysophyllum albidum* fruit were 1.35 mg/mL and 76.35 U/mg protein, respectively, while 0.925 mg/mL and 1.59 U/mg protein were respective values for unripe *Chrysophyllum albidum* fruit (Table 2). The ripe *Chrysophyllum albidum* fruit had more enzyme activity than the unripe, hence, all analysis was carried out using the ripe fruit.

In unripe fruit, the activity of pectinase is low as compared to the ripe. During ripening, the enzymes break the pectin backbone and alter its structure resulting in a more soluble molecule.[2,8] A purification fold of 4.13 and 41.81 was observed using ammonium sulphate precipitation and gel filtration, respectively, for polygalacturonase of ripe *Chrysophyllum albidum* fruit as shown in Table 3. Figures 1 and 2 shows the elution fractions of protein and polygalacturonase activity, respectively, using gel filtration. Polygalacturonase activity showed a major peak at fractions 18, 21, 22, 23 and 24, hence, these five fractions were pooled together and assessed for their kinetic properties. The enzyme activity observed at four peaks may suggest the presence of isoenzymes with different molecular weights. It is known that gel chromatography separates proteins according to their molecular weights, further corroborating this finding.[20] The effects of different pH (3.0 - 9.0) on polygalacturonase activity of *Chrysophyllum albidum* fruit extract is shown in Figure 3. Four activity peaks (pH 5.0, 6.0, 6.5 and 7.5) were obtained for the enzyme. The enzyme was more active in an acidic environment. The polygalacturonase enzyme of *Chrysophyllum albidum* fruit has a broad pH range between pH 5.0 and 7.5, probably due to different isoenzyme forms. In this study, the purification fold of polygalacturonase using gel filtration was lower while that of ammonia precipitation was higher than that reported for *S. aethiopicum*.[20] The broad pH activity peaks would suggest that the enzyme can withstand variations in pH.[17] However, the optimum pH of 5.0 implies the enzyme was more active in an acidic environment, hence, *Chrysophyllum albidum* fruit produces acidic polygalacturonase that may be suitable for fruit juice clarification.[21] This finding is in agreement with previous reports on the acidic nature of their extracted polygalacturonase.[21-23] Figure 4 shows the optimum temperature of polygalacturonase activity of *Chrysophyllum albidum* fruit extract was 40°C. *Chrysophyllum albidum* fruit polygalacturonase will likely be effective between 40 – 60°C as a result of over 50% activity observed in these temperature ranges. This property may be desired for industrial processes where high temperatures are a common occurrence. A study by Aminzadeh,[24] reported 40°C as the optimal temperature for polygalacturonase produced by *Tetracoccosporium* species corroborating this study. Contrary to this study, the optimal temperature of polygalacturonase from *Solanum macrocarpum* fruit was reported to be 30°C.[20] The polygalacturonase activity at the different incubation time intervals of *Chrysophyllum albidum* fruit extract is shown in Figure 5. Very high enzyme activity was observed in the first 10 minutes, which was relatively constant in the subsequent time range. The activity of polygalacturonase in *Chrysophyllum albidum* fruit at varying incubation periods showed a rapid release of the product that remained stable. The stable release of products at different time frames would mean the polygalacturonase from *Chrysophyllum albidum* fruit is stable and may not be inhibited by feedback mechanism in a relatively large amount of product. The effect of different substrate concentrations on polygalacturonase activity of *Chrysophyllum albidum* fruit extract revealed a continuous increase of enzyme activity as the substrate concentration increased (Figure 6). The Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) for polygalacturonase obtained from the Lineweaver-Burk plot is 3.97 mg/mL and 81.82 U/mg protein, respectively (Figure 7). A continuous increase in enzyme activity was observed as the substrate concentration increased. Since a hyperbolic curve was not observed, this implies that the enzyme had not yet reached saturation. This finding was further buttressed by the V_{max} and K_m obtained in the Lineweaver-Burk plot.

Table 1: Characteristics of *Chrysophyllum albidum* fruit

Name	State	Exocarp colour	Mesocarp colour	Texture	Seed	Fruit shape
Chrysphyllum albidum	Unripe	Green	Green	Hard	Semi oval shaped	oval
	Ripe	Orange	Orange	Soft	Semi oval shaped	oval

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Table 2: Protein concentration and polygalacturonase activity of *Chrysophyllum albidum* fruits

	Ripe *C. albidum*	Unripe *C. albidum*
Total Protein Concentration (mg/mL)	1.35	0.925
Polygalacturonase activity (U/mg protein)	76.35	1.59

Table 3: Purification table for polygalacturonase of *Chrysophyllum albidum* fruit

Purification step	Volume (mL)	Total Activity (U/mL)	Total Protein (mg/mL)	Specific Activity (U/mg protein)	Yield (%)	Purification Fold
Crude Extract	100	10307.83 ± 3.91	135 ± 0.04	76.35 ± 0.43	100	1
Ammonium sulphate (80%)	20	5744.70 ± 2.55	3.64 ± 0.01	315.64 ± 14.03	55.73	4.13
Gel Filtration (Sephadex G-100)	5	6066.00 ± 2.90	< 0.1	3192.5 ± 151.79	58.85	41.81

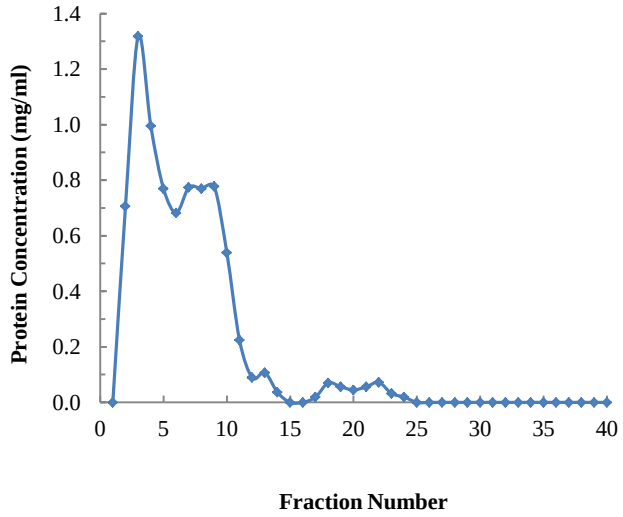

Figure 1: Elution profile of proteins of *Chrysophyllum albidum* fruit (Sephadex G-100)

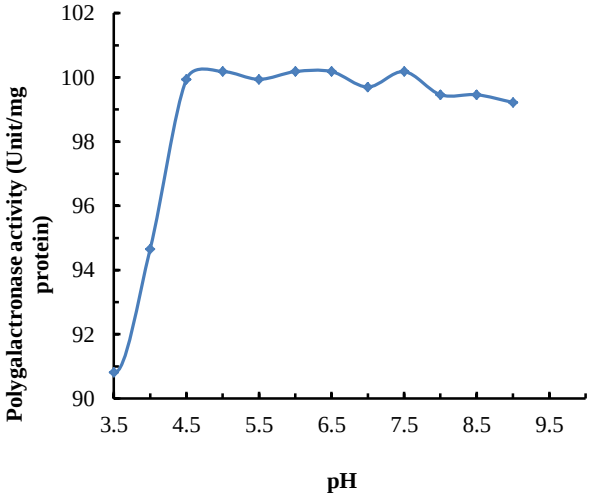

Figure 3: Effect of pH on the polygalactrounase activity of *Chrysophyllm albidum* fruit

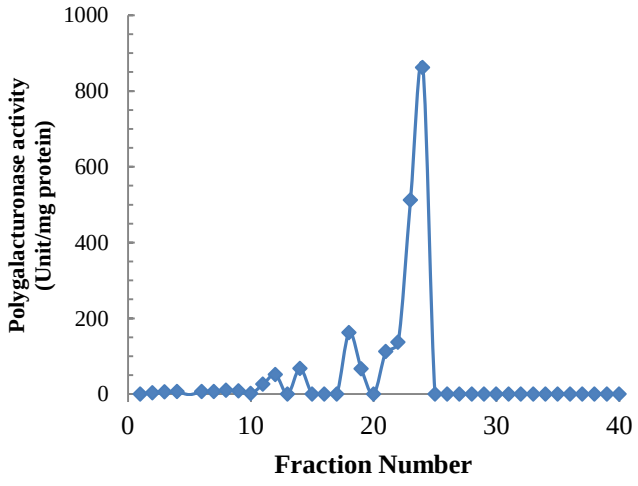

Figure 2: Elution profile of polygalacturonase of *Chrysophyllum albidum* fruit (Sephadex G-100).

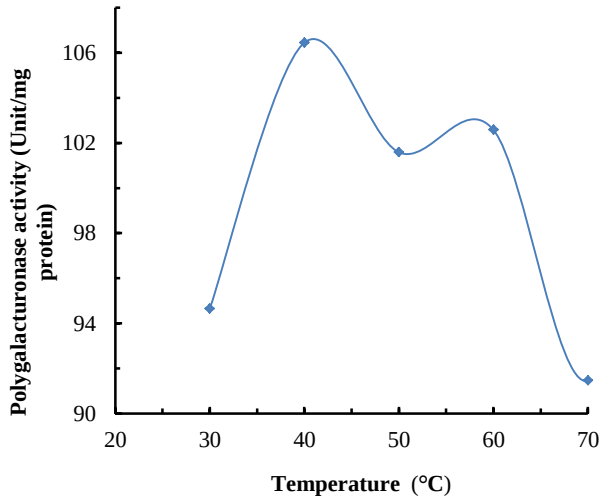

Figure 4: Effect of temperature on the polygalactrounase activity of *Chrysophyllm albidum* fruit.

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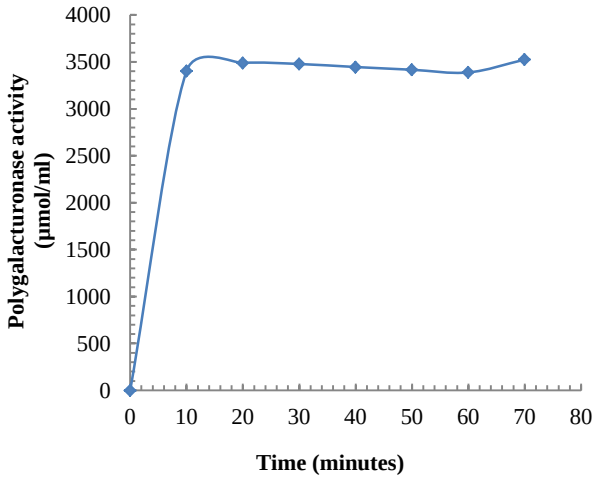

Figure 5: Time course of the polygalacturonase activity of *Chrysophyllm albidum* fruit.

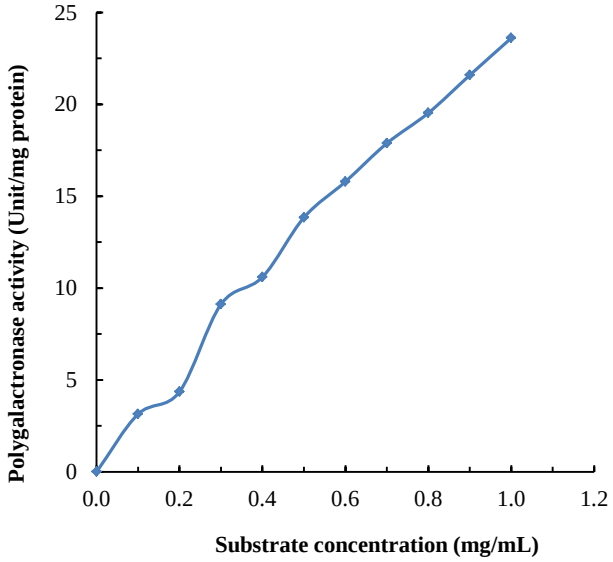

Figure 6: Effect of substrate concentration on the polygalacturonase activity of *Chrysophyllm albidum* fruit.

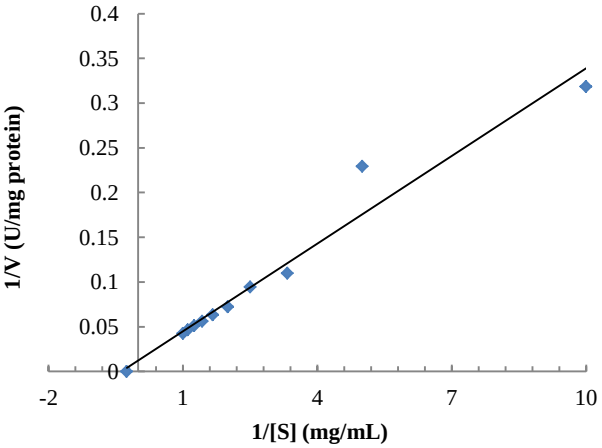

Figure 7: Lineweaver-burk plot of polygalacturonase activity of *Chrysophyllum albidum* fruit

Conclusion

The result of this research has proven the presence of polygalacturonase in *Chrysophyllum albidum* fruit, with a good prospect of producing the enzyme from the fruit. The enzyme functioned best at acidic pH and 40°C. However, characterization, thermal behaviour, optimisation and industrial application of the enzyme can be further researched on for local production.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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