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Isolation of Carbohydrate Hydrolyzing Enzymes from Red Kidney Beans

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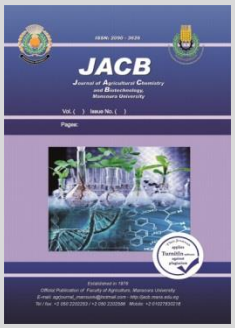

ABSTRACT

Humans and other monogastric animals lack certain glycosidase enzymes that are widely present in plant seeds and animal tissues. This study aimed to extract glycosidase enzymes from red kidney bean seeds. The optimal germination period was found to be 6 days, resulting in the extraction of approximately 230.0 g of cotyledons. Analysis revealed the presence of six forms of glycosidase enzymes: α- and β-glucosidase, α- and β-galactosidase, and α- and β-mannosidase. The protein concentration in the extract was determined to be 0.271 mg protein ml^{-1}. Enzyme activities were measured for each form: α-glucosidase (1.90 mM min^{-1} ml^{-1}), β-glucosidase (4.10 mM min^{-1} ml^{-1}), α-galactosidase (0.76 mM min^{-1} ml^{-1}), β-galactosidase (1.70 mM min^{-1} ml^{-1}), α-mannosidase (2.07 mM min^{-1} ml^{-1}), and β-mannosidase (0.90 mM min^{-1} ml^{-1}). Specific activities were also calculated: α-glucosidase (3.76 mM min^{-1} mg $protein^{-1}$), β-glucosidase (8.10 mM min^{-1} mg $protein^{-1}$), α-galactosidase (1.55 mM min^{-1} mg $protein^{-1}$), β-galactosidase (3.31 mM min^{-1} mg $protein^{-1}$), α-mannosidase (4.25 mM min^{-1} mg $protein^{-1}$), and β-mannosidase (1.90 mM min^{-1} mg $protein^{-1}$). Study highlights the high activity levels of carbohydrate hydrolysis enzymes (glycosidases). In red kidney bean plants. The findings emphasize the importance of red kidney bean seeds as rich source of these biocatalysts with diverse applications.

Keywords: Glycosidases, cotyledons, protein

INTRODUCTION

Glycosidase enzymes-are essential for breaking down carbohydrates into simpler forms that can be easily absorbed and utilized by organisms (Morant *et al*., 2008 and Cairns and Esen, 2010).-They are found in various plant seeds and animal tissues, but some species, like humans and other monogastric animals, lack certain glycosidases. This deficiency has led to research on identifying alternative sources of these enzymes to address dietary and nutritional requirements. (Sowbhagya and Chitra, 2010 and Marathe *et al*., 2017). Red kidney beans (*Phaseolus vulgaris*) are rich in glycosidase enzymes, which make them a valuable source for enzyme extraction and research. Studying the properties and functions of these enzymes can offer valuable insights for their potential use in industries such as food processing, pharmaceuticals, and biotechnology (Zhang *et al.*, 2008). This research aims to extract and characterize different forms of glycosidase enzymes from red kidney bean seeds. The goal is to isolate and analyze these enzymes—to determine their specific activities and roles in carbohydrate metabolism. Additionally, the study will investigate optimal conditions for enzyme extraction and activity, including the germination period of red kidney beans. The findings will contribute to understanding the glycosidase enzymes in red kidney beans and their potential applications in various fields.

MATERIALS AND METHODS

1.Chemicals and raw materials.

The enzyme substrates, including p-nitrophenyl-α- and β-D-glucopyranoside, p-nitrophenyl α- and β-D-glactopyranoside, and p-nitrophenyl α- and β-D-mannopyranoside, were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Red kidney bean seeds of the "*Phaseolus vulgaris* L. Cv Sharmili" variety were obtained from the agricultural research center in Egypt.

2.*Crude enzymes.*

The enzyme crude extract was prepared following the method described by Agrawal and Bahl (1968) and Zhang *et al.*, (2008), in three main steps. Initially, red kidney bean seeds (approximately 230.0 g) were germinated at 28°C in darkness for 6 days, after which the cotyledons were separated. Next, the cotyledons were washed and treated with 0.20 M sodium citrate buffer at pH 6.00 in a 1:1.5 ml ratio. The clear supernatant was collected and the sediment was discarded. Finally, fractionation with ammonium sulfate was carried out by adding 430.0 g per 1000 ml of solid ammonium sulfate to the supernatant. Stirring was continued for 45 to 60 minutes to achieve 65% saturation.

3. The enzyme crude extract obtained from the investigation was subjected to further analysis.

Enzyme Assays:

Enzyme activity was measured according to the procedure outlined by Bulens *et al.* (2011). Each enzyme was assayed at its optimal pH in 0.05 M citrate-phosphate buffer. A unit of enzyme activity was defined as the amount capable of releasing 1.0 mmole of p-nitrophenol per minute at 30°C. Specific enzyme activity was calculated as units per milligram of protein. The amount of p-nitrophenol released during the assay was determined by referencing a calibration curve prepared simultaneously, using concentrations ranging from 0.10 to 1.00 mM of p-nitrophenol.

Detection of glycosidases.

A qualitative analysis of the crude extract from red kidney beans was performed to detect glycosidase enzymes, following the method described by Bahl and Agrawal (1968); Dignum *et al.*, (2001).

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Protein concentration:

The protein concentrations in the crude enzyme extract of red kidney beans were determined spectrophotometrically, following the method described by Kalckar (1947). Measurements were made at wavelengths of 260 nm and 280 nm, and protein concentrations were calculated in milligrams per milliliter using the equation.

mg protein ml $^{-1}$ = [1.45 (E_{280} nm) - 0.74 (E_{260} nm)]

Kinetic studies:

Enzyme activity of glycosidases were measured using a synthetic substrate concentration of 3.0 mm, instead of the standard 25 mM, and quantified as mm/min/ml, following the method described by Li and Li (1970). The influence of pH on enzyme activity was investigated using p-nitrophenyl glycosides and a citrate-phosphate buffer solution at 0.05 M with pH values ranging from 3.50 to 6.50. Enzyme activity was assessed at different pH levels. Furthermore, the impact of substrate concentration on enzyme activity was investigated by varying the substrate concentrations (ranging from 3.0 to 30.0 mM) under optimal conditions.

RESULTS AND DISCUSSION

In this study, the extraction and fractionation of germinated red kidney bean seeds were conducted at room temperature. The findings indicate that red kidney beans contain active glycosidases, such as α- and β-glucosidase, α- and β-galactosidase, and α- and β-mannosidase, in highly active forms. The enhanced activity of these enzymes may be linked to the germination process of the seeds. The germination process enhances enzyme activity in red kidney bean seeds by degrading proteins and polysaccharide gums. These gums form a viscous solution in water, which can complicate purification steps if not removed early on. In our isolation scheme, process, we remove polysaccharides through germination and repeated precipitations with ammonium sulfate. Fractionation with acetone or alcohol initially is not ideal, as both polysaccharides and proteins would precipitate together.

In the investigation, key steps were taken to extract and analyze glycosidases from red kidney bean seeds. Initially, seed germination was optimized to occur over 6 days, yielding approximately 230.0 g of cotyledons. Extraction followed, resulting in a volume of 375 ml after squeezing and centrifugation. Ammonium sulfate fractionation was then carried out by subjecting the extract to 65.0% saturation, with 161.50 g of ammonium sulfate added. The process was repeated, and the resulting precipitate was dissolved in sodium citrate buffer, (pH 6.00,) and further fractionated using citrate buffer, (pH 4.60). A crude enzyme extract was obtained by precipitating with ammonium sulfate, followed by dialysis and centrifugation. The crude extract was then analyzed for glycosidase presence and protein concentration.

Table 1 shows the presence of different glycosidases in red kidney bean extract, such as α- and β-glucosidase, α- and β-galactosidase, and α- and β-mannosidase. This confirms that red kidney bean seeds and tissues contain a variety of glycosidases that can be used to break down carbohydrate components of glycoproteins and polysaccharides. Additionally, the protein concentration in the crude extract was found to be 0.271 mg protein per ml. This analysis indicates that red kidney bean seeds contain high levels of glycosidases, which play a crucial role in carbohydrate metabolism and have potential industrial uses.

Table 1. Glycosidase content in red kidney bean crude extract.

Enzymes	Present or absent	Protein concentration (as mg protein ml^{-1})
α- glucosidase	+	0.271
β – glucosidase	+	
α- galactosidase	+	
β – galactosidase	+	
-α- mannosidase.	+	
β – mannosidase.	+	

+ = Present; - = Absent

Numerous studies, such as Nevins (1970), have shown the high activity of glycosidases extracted from red kidney bean seeds. These enzymes, including α- and β-glucosidase, α- and β-galactosidase, and β-xylosidase, have been effectively isolated. Our research , as well as studies by other scholars, confirm the widespread presence of carbohydrate hydrolysis enzymes (glycosidases) in various plant parts such as seeds, leaves, and roots. These enzymes play a crucial role in breaking down polysaccharides in cell wall glycoproteins, releasing monosaccharides.

In summary, the presence of glycosidases in red kidney bean seeds is significant. The protein concentration in the crude enzyme extract of red kidney beans was found to be 0.271 mg protein ml-1, with absorbance readings of 0.855 at E280 and 1.309 at E260.

Glycosidases characterization:

Enzyme activity was assessed using the method outlined by Li and Li (1970), quantifying activity in micromoles of p-nitrophenol. Table 2 shows the correlation between different concentrations of p-nitrophenol and their optical densities at 420 nm, based on the standard curve. Table 3 displays the enzyme activities and specific activities of α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase, and β-mannosidase. The increase in substrate concentrations led to higher enzyme activity and specific activity. Table 4 shows that varying pH values (3.50, 4.50, 5.50, and 6.50) did not significantly affect enzyme activity. Table 5 demonstrates that enzyme activity and specific activity for α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase, and β-mannosidase increased with substrate concentration (3.0, 6.0, 12.0, and 30.0 mM).

Table 2. Relation among various concentrations of p-nitrophenol and its optical density for standard curve

Concentration(mM/ml)	OD (420 nm)
0.10	0.069
0.20	0.180
0.30	0.225
0.40	0.322
0.50	0.399
0.60	0.472
0.70	0.525
0.80	0.635
0.90	0.699
1.0	0.775

Table 3. Enzyme activity (mM min^{-1} ml^{-1}) and specific activity (mM min^{-1} mg protein^{-1}) of the investigated glycosidases

Enzymes	Enzyme activity	Specific activity
α- glucosidase	1.90	3.77
β – glucosidase	3.70	8.11
α- galactosidase	0.76	1.58
β – galactosidase	1.70	3.29
-α- mannosidase	2.07	4.28
β – mannosidase	0.90	1.92

These results emphasize the interplay between substrate concentration and enzyme kinetics in red kidney bean glycosidases.

Table 4. Effect of pH on the enzyme activity (mM min^{-1} ml^{-1}) and specific activity (mM min^{-1} mg $protein^{-1}$) of the investigated glycosidases

Enzymes	Enzyme activity				Specific activity			
	pH (3.5)	pH (4.5)	pH (5.5)	pH (6.5)	pH (3.5)	pH (4.5)	pH (5.5)	pH (6.5)
α- glucosidase	1.907	1.930	1.900	1.932	3.76	3.79	3.82	3.70
β – glucosidase	4.10	4.15	3.98	3.98	8.10	8.10	8.15	8.10
α- galactosidase	0.76	0.79	0.74	0.75	1.55	1.58	1.59	1.60
β – galactosidase	1.70	1.72	1.73	1.70	3.31	3.30	3.29	3.25
-α- mannosidase	2.07	2.08	2.05	2.06	4.25	4.25	4.30	4.32
β – mannosidase	0.92	0.91	0.90	0.90	1.90	1.95	1.93	1.90

Table 5. Effect of concentration of substrate on the enzyme activity (mM min^{-1} ml^{-1}) and specific activity (mM min^{-1} mg $protein^{-1}$) of the investigated glycosidases

Enzymes	Enzyme activity				Specific activity			
	S(3.0 mM)	S(6.0 mM)	S(12.0 mM)	S(30.0 mM)	S(3.0 mM)	S(6.0 mM)	S(12.0 mM)	S(30.0 mM)
α- glucosidase	0.60	0.75	0.82	1.05	1.08	1.55	1.78	1.85
β – glucosidase	1.38	1.45	1.55	1.68	2.15	2.70	3.10	3.45
α- galactosidase	1.65	1.78	1.88	2.25	3.9	4.2	4.25	4.40
β – galactosidase	2.85	3.01	3.15	3.85	5.90	6.13	7.45	8.00
-α- mannosidase	2.055	2.13	2.45	3.00	4.30	4.38	4.78	5.12
β – mannosidase	0.645	0.750	1.17	1.40	1.34	1.78	2.45	3.12

CONCLUSION

This study systematically investigated the enzymatic properties of glycosidases extracted from germinated red kidney bean seeds. Our experiments revealed robust enzyme activity under various conditions, such as different pH levels and substrate concentrations. The results indicate that red kidney bean seeds are rich sources of glycosidases, including α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase, and β-mannosidase. Interestingly, we observed a positive correlation between enzyme activity and specific activity with substrate concentration suggesting substrate-dependent enzymatic kinetics.The consistent enzymatic activity of glycosidases across different pH levels indicates a lack of pH dependency .This finding enhances our knowledge of the biochemical properties of glycosidases in red kidney bean seeds and highlights their potential for industrial and biotechnological uses. The study reveals insights into the enzymatic behavior of glycosidases and emphasizes the importance of red kidney bean seeds as a rich source of these versatile biocatalysts.

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عزل إنزيمات التحلل الكربوهيدراتي من الفاصوليا الحمراء

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الملخص

يفتقر الانسان والحيوانات أحادية المعدة الأخرى إلى إنزيمات جليكوسيداز معينة موجودة على نطاق واسع في بذور النباتات والأنسجة الحيوانية. ولذلك كان الهدف من هذا البحث هو استخلاص أشكال مختلفة من إنزيمات الجليكوسيداز من بذور الفاصوليا الحمراء. وجدت الدراسة أن فترة الإنبات الأمثل للفاصوليا الحمراء كانت ٦ أيام، مما أدى إلى استخلاص ما يقرب من ٢٣٠,٠ جرام من الفلقات. أظهر تحليل الاستخلاص وجود ستة أشكال من إنزيمات جليكوسيداز: ألفا-وبيتا-جلوكو سيداز، ألفا- وبيتا-جالاكتوسيداز، وألفا- وبيتا-مانوسيداز. تم تحديد تركيز البروتين في الاستخراج بواقع ٠,٢٧١ ملجم بروتين مل$^{-1}$. تم قياس أنشطة الإنزيم لكل نوع: ألفا-جلوكوسيداز (١,٩٠ ملمول دقيقة$^{-1}$ مل$^{-1}$)، بيتا-جلوكوسيداز (٤,١٠ ملمول دقيقة$^{-1}$ مل$^{-1}$)، ألفا-جالاكتوسيداز (٠,٧٦ ملمول دقيقة$^{-1}$ مل$^{-1}$)، بيتا-جالاكتوسيداز (١,٧٠ ملمول دقيقة$^{-1}$ مل$^{-1}$)، ألفا-مانوسيداز (٢,٠٧ ملمول دقيقة$^{-1}$ مل$^{-1}$)، وبيتا-مانوسيداز (٠,٩٠ ملمول دقيقة$^{-1}$ مل$^{-1}$). تم حساب الأنشطة الخاصة أيضًا: ألفا-جلوكوسيداز (٣,٧٦ ملمول دقيقة$^{-1}$ ملجم بروتين$^{-1}$)، بيتا-جلوكوسيداز (٨,١٠ ملمول دقيقة$^{-1}$ ملجم بروتين$^{-1}$)، ألفا-جالاكتوسيداز (١,٥٥ ملمول دقيقة$^{-1}$ ملجم بروتين$^{-1}$)، بيتا-جالاكتوسيداز (٣,٣١ ملمول دقيقة$^{-1}$ ملجم بروتين$^{-1}$)، ألفا-مانوسيداز (٤,٢٥ ملمول دقيقة$^{-1}$ ملجم بروتين$^{-1}$)، وبيتا-مانوسيداز (١,٩٠ ملمول دقيقة$^{-1}$ ملجم بروتين$^{-1}$). في الختام، يستضيف نبات الفاصولياء الحمراء أشكالًا نشطة للغاية من إنزيمات تحلل الكربوهيدرات (الجليكوسيداز). بشكل عام، يسلط هذا البحث الضوء على الديناميات الإنزيمية للجليكوسيداز ويؤكد على أهمية بذور الفاصولياء الحمراء كمصدر قيم لهذه الإنزيمات الحيوية ذات التطبيقات الم