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EXTRACTION AND PURIFICATION OF LACCASE ENZYME FROM TERMITE GUTS

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ABSTRACT

This study was conducted at the University of Baghdad, College of Agricultural Engineering Sciences, with the aim of extracting laccase enzyme from the bowels of termites. The concentration method was used by precipitation (with ammonium sulfate) with gradient saturation rates from 30% to 80%. The results showed that the highest effectiveness was at the saturation rate of 80%, reaching 5.991 units/mg, dialysis reaching 3.763 units/mg, and gel filtration reaching 7.644 units/mg. m and ion exchange amounted to 5.867 units/mg. The results of purification by gel filtration showed one distinct protein peak, and the ion exchange chromatography technique was used, as three protein peaks appeared, peak (1) representing the protein, and peak (2) representing the bound protein and it represents the laccase enzyme. After measuring the activity, one peak appeared, with an enzyme yield of 32.192. % and the number of purification times is 15,355.

Keywords: Laccase, Extraction, Termite guts, Purification.

أستخلاص وتنقية انزيم LACCASE من أحشاء حشرة الارضة

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الخلاصة

أجريت هذه الدارسة بجامعة بغداد كلية علوم الهندسة الزراعية بهدف استخلاص أنزيم laccase من أحشاء حشرة الارضة، استعملت طريقة الترسيب التركيز(بكبريتات الأمونيوم) بنسب إشباع متدرجة من 30% إلى 80%، وأظهرت النتائج ان أعلى فعالية كانت عند نسبة الإشباع 80% اذ بلغت 5.991 وحدة/ مل، والترشيح الهلامي بلغت 7.644 وحدة/ ملغم و التبادل الأيوني بلغت 5.867 وحدة/ مل . كما اظهرت نتائج التنقية بالترشيح الهلامي قمة بروتينية واحدة متميزة، واستعملت تقنية كروماتوكرافيا التبادل الأيوني اذ ظهرت ثلاث قمم من البروتين، تمثل القمة (1) البروتين، والقمة (2) تمثل البروتين المرتبط وهي تمثل انزيمLaccase بعد قياس الفعالية اذ ظهرت قمة واحدة وبحصيلة إنزيمية 32.192% وعدد مرات التنقية 15.355.

الكلمات المفتاحية: اللاكيز، أستخلاص، أحشاء حشرة الارضة، تنقية.

[*]**The article is taken from the doctoral thesis of the first researcher.**

INTTRODUCTION

Microcerotermes versus silva is one of the species widespread in Iraq. It is an insect that lives in communities called colonies estimated to number from 30,000 to 2.5 million insects. (**Fagbohunka *et al*., 2016; Khan *et al*., 2020)** The termite is highly efficient in feeding and is very effective in breaking down lignocellulose **(Khan *et al*., 2020).**This difficult work is accomplished by microorganisms that live in the hindgut of the insect called Protozoa The insect predominantly harbors bacteria as it has a huge diversity of enterobacteria, protozoa, some archaea, some fungi, and some phages in its gut that provide nourishment to its host as it feeds mainly on plant biomass, which is a diet rich in cellulose, hemicellulose, and lignin, making each termite gut a unique storehouse of encoded genes for industrially viable enzymes (**Marynowska *et al.*, 2017**), the degradation of lignocellulose in termites relies on a dual system that involves the activity of both the insect (mechanical and enzymatic digestion) and the microbiome. Yin and carbohydrates. Microbial enzymes play a synergistic role with endogenous enzymes in the host insect for the mass degradation of lignocellulose (**Geng *et al.*,2018**).The guts of termites contained enzymes from the same insect and existing microbiological enzymes (**De Albuquerque *et al.*,2014**) identified many kinase enzymes involved in the degradation of lignin mainly as laccase s, lignin peroxidase and manganese peroxidase, aldo-keto reductases and catalases (**Egwim *et al.*,2015**), laccase enzyme was produced from xylophagic bacteria isolated from termites,six positive strains were isolated, and laccase was produced by these bacterial isolates at37C(**Hassan *et al.*,2018**)and pH 5.5, 6.2 and 7.0. Termite guts produce extracellular laccase with activity at moderate to moderate pH (**Yasamin *et al* ., 2022; Maia *et al.*,2021**) enzymes biotechnological applications have been the subject of research for more than 30 years and this research has covered many disciplines such as the food industry, paper industry, cosmetics, pharmaceutical industry, textile industry, and many other industries (**Awda & Khalifa, 2019**) and has a role in the degradation of lignin in ruminant feed (**Wafaa *et al*. 2022; Al-Samaraae& Alwaeli., 2016**). Enzyme is an effective additive with agricultural industrial by-products, to improve the nutritional value of high-fiber diets. Therefore, extracellular enzymes are used to hydrolyze lignocellulose in many animal production systems. Therefore, animal nutritionists have tried to improve feeds through these exogenous enzymes, as these are not digested. Feed from the animal efficiently and supplementing the feed with specific enzymes increases the nutritional value of the feed ingredients and improves the digestion efficiency of the ruminant animal **(Jassem *et al*., 2020; Hassan *et al.*, 2008**).

MATERIALS AND METHODS

Collecting termites and extraction of the laccase enzyme

Termites were obtained from Diyala orchards, washed with distilled water, and homogenized in a sodium acetate buffer solution at a concentration of 0.01 M and pH = 5. The solution contained 1 mmol of Ethylene diamin etetra acetic acid EDTA, then the insects were mashed with an electric blender. The centrifugation process was carried out at a speed of 10000/**x**g for 10 minutes the filtrate is taken. The enzyme is extracted if it is filtered using cotton, followed by a second filtration step using filter paper, and the extract that represents the crude enzyme extract that determines its size, activity and protein concentration in it, and keep in the refrigerator until use. The rotein concentration was estimated according to the Lowry method modified by **Cooper (1977)**.

Determination of the activity of Laccase enzyme

The determination of the activity of the Laccase enzyme was carried out using the Guaiacol Assay Method described by (**Desai *et al.*, 2011**).

Extraction and purification of laccase enzyme

The process of concentrating the laccase enzyme was carried out by precipitating it with ammonium sulfate at a saturation rate of 80%. Then cooling centrifugation was carried out at a speed of 10000 **x**g. Against a solution of EDTA with a concentration of 0.01 M and a pH of (5) for a period of 24 hours, during which time the buffer solution was replaced three times, and after extracting the dialysis strip, it was placed in a bowl containing sugar (table sugar) to remove excess water(dialysis). Then the gel filtration was done, as Sephadex 100G were mixed with distilled water and placed in a water bath at a temperature of 85-90ºC for 3 hours with continuous stirring, then left to cool in the air. After the stagnation of the gel, get rid of the upper liquid quietly. Then, the mixture was filled in a glass column to give a gel with dimensions (60 x 1.5 cm). The column was equilibrated using NaCl saline. Six beakers were prepared, each beaker containing 80 ml of NaCl saline at concentrations (0.1, 0.25, 0.5, 0.75, 1), which were used respectively to wash the gel column, then the concentrated enzyme was added from the dilution step. To a column, 100 ml of Sodium acetate buffer was added, and the parts were collected, each part containing 5 ml. The absorbance of each part was measured at 450 nm, then the enzyme activity was estimated in each part. The parts with enzyme activity were collected, and the size, enzymatic activity, and protein concentration were estimated (**Imran, 2015**), then ion exchange filtration was performed using 20 grams of diethyl amino ethyl-cellulose (DEAE-cellulose) powder on s in 1000 mL of distilled water and allowed to settle, the supernatant was discarded, and this step was repeated several times until it became clear. DEAE-cellulose was then activated with 0.25 HCl for 30 min, filtered with a Buchner funnel containing Whatman No. 1 filter paper and washed with distilled water twice (**Whitaker&Bernhard., 1972**), after DEAE-cellulose was activated with 0.25N NaOH, the filtration and washing processes were repeated twice. Substance M was titrated EAE-cellulose activated with Sodium acetate buffer (pH5), and filled in a column with dimensions (2.5 x 16) cm. The concentrated enzyme from the dialysis step was added to the ion exchange column. 100 ml of Sodium acetate buffer was added and the flow rate was regulated to be 0.8 ml/min for the washed and filtered samples, which were collected as 5 ml per portion. The absorbance of each portion was measured at 450 nm for enzyme activity and protein concentration (**Imran,2015**).

RESULTS AND DISCUSSION

Table (1) shows the steps for purify the Laccase enzyme produced from the ground insect. In the first step, the specific activity of the enzyme in the crude protein was 2.894 (units/mg), and then the highest enzymatic yield was chosen, reaching 65.748%, for later approval in the subsequent purification step. Ammonium sulfate was used to precipitate the enzyme, and the best saturation percentage for precipitating the enzyme was (80%). then a dialysis tube was used 60-70 KD a depending on the molecular weight of the enzyme **(Janusz et al., 2020),** (**Janusz *et al.*, 2020**). The results indicate the presence of the Laccase enzyme **(AI-Ani, 2005).**

Table (1): Laccase enzyme purification steps.

Purification step	Volum (ml)	Activity (unit/ml)	Protein (mg/ml)	Total activity (unit)	Specific activity unit/mg	The Purification fold	Enzyme yield (%)
Crude enzyme	62	4.409	1.523	273,358	2.894	1	100
ammonium sulfate	30	5.991	1.405	179.73	4.264	1.433	65.748
gel filtration	35	3.763	0.103	131.705	36.533	12.623	48.180
ion exchange	15	5.867	0.132	88.00	44.440	15.355	32.129

after the dialysis process, the gel filtration chromatography technology was used using a gel filtration column (Sephadex-G100. Figure (1) shows the results of purification by gel filtration, as one distinct protein peak appears, and when measuring the enzymatic activity, one peak appeared confined between tubes 13-19, with an enzyme yield of 55.926% and the number of purification times 14.124.

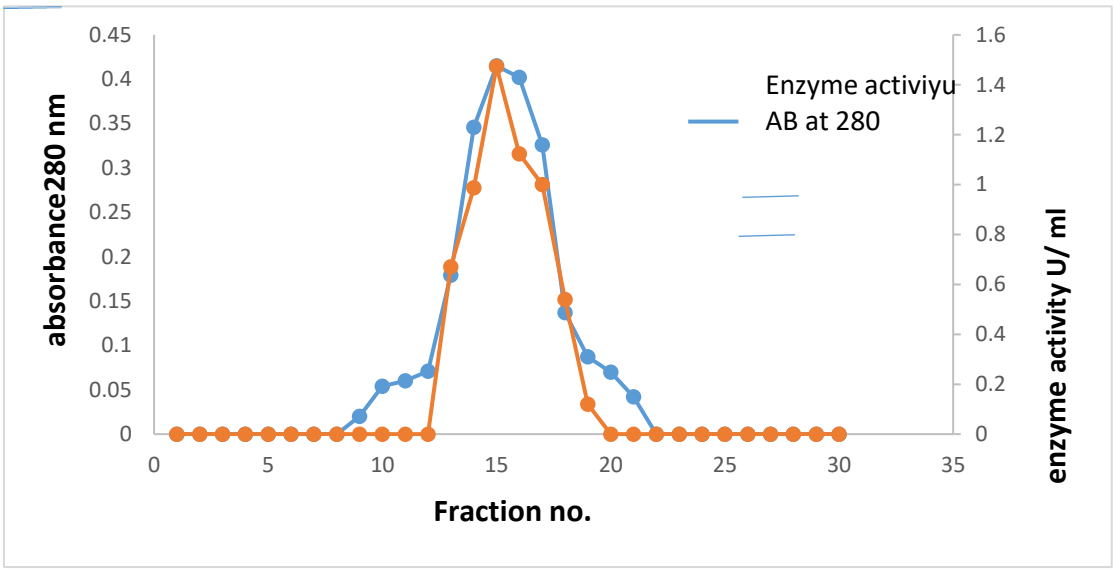

Fig (1) Purification of the laccase enzyme extracted from the bowels of termites by gel filtration method using a Sephadex G-100 column with dimensions of (1.5 x 60) cm.

The ion exchange chromatography technique was used using the ion exchange (DEAE-Sephadex A20). Figure (2) shows the results of purification in this way, as three protein peaks appeared. Peak (1) represents the non-column-bound protein represented by parts confined between 9-14, and peak (2) represents the bound protein represented by the recovered parts confined between 28-38. It represents the Laccase enzyme after measuring the activity as one peak appeared. Figure 33- 35, with an enzyme yield of 32.192%, the number of purification times 15.355, and the peak (3) represents the bound protein and does not have any enzymatic activity **(Hussain., 2011).**

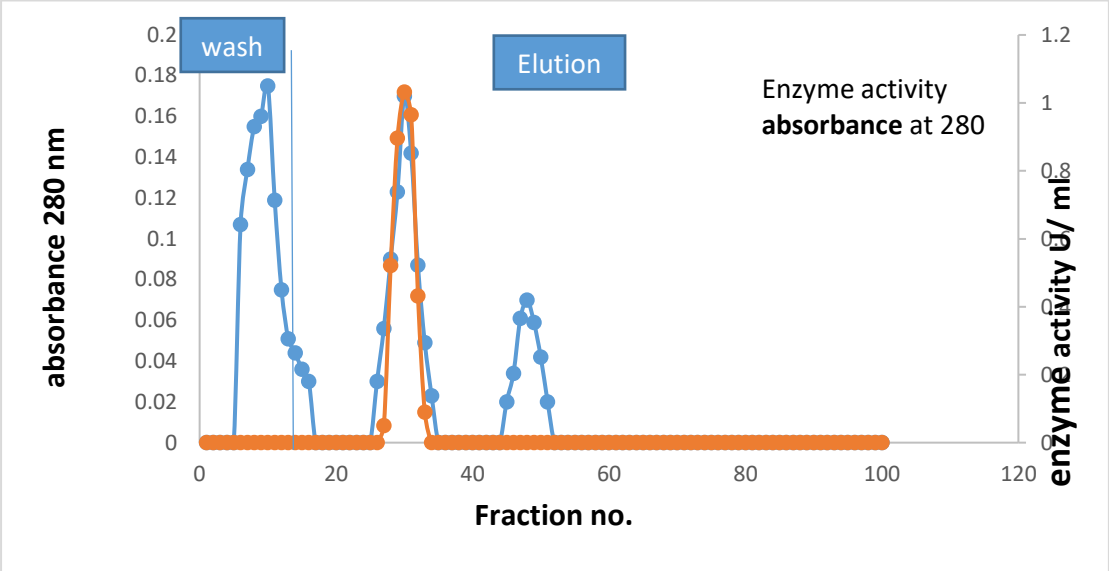

Fig (2): purification of the laccase enzyme by ion exchange method using the DEAE-Sephadex A20 ion exchanger in a column with dimensions of (2.5 x 16) cm, at a running speed of 60 ml/h and at a rate of 5 ml/part.

CONCLUTIONS

In practice, this study can obtain the exogenous enzyme Laccase From the entrails of ground insects and purify them using one of the modern techniques, The ion exchange chromatography technique used using the ion exchanger shows the results of one protein peak It represents the Laccase enzyme after measuring the activity which can be used to improve rough feed. Rich in fiber the use of enzymes in animal feed appears to be a safe method in the food supply chain for consumers.

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