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Study of Extraction, Purification and Characterization of Urease Enzyme from some plant Leguminales

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ABSTRACT

The present study included investigation of the urease enzyme in plant extracts belonging to the legume family, which included plant seed extracts (*Vigna unguiculata, Vicia faba, Phaseolus vulgaris and Lagonychium farctum*). The results showed that the highest enzyme efficacy of the urea was in the extract of the chickpea seeds and was chosen as a source for the study of the enzyme. The urease was investigated in parts of the chickpeas (seeds, stems and leaves). The highest enzyme activity and quality of the urea were in seed extract *Lagonychium farctum*. The optimum conditions for enzyme extraction were determined using different pH and temperature solutions. The potassium phosphate solution at 0.02 µl and pH 8 was best suited for enzyme extraction where the enzyme recorded an enzyme activity of 3.195 U / ml. The best reaction time for enzyme extraction was 30 min, giving an enzyme efficacy of 3.274 U/ml. The ionization process was purified by several steps including ammonium sulphate deposition with different precipitation ratios. The best saturation was 50%. The ionic-cellulose ionization was then filtered with the gel filtration on the Sephadex G-200 column. The filtration times were 29.6 times and with an enzyme yield of 75%.

Keywords: Purification, Extraction, EC.3.5.1.5, Urease and Leguminales.

1. INTRODUCTION

Urease (Urea amidohydrolase EC.3.5.1.5) a nickel-based metal enzyme (Ni^{+2}) to stimulate the hydrolysis of urea at a rate of 1014 times faster than the non-stimulating reactions producing ammonia and carbon dioxide[1-7] [4]. Urease produces a large number of organisms, including high-end plants and microorganisms, which are of great importance in biotechnology, clinical chemistry, immunochemistry and medical fields, as it is associated with certain human diseases such as urinary tract diseases, gastric ulcers and duodenum If not treated to cancer. Thus, knowledge of the mechanism of urea work as a ferment factor for the bacteria causing the diseases mentioned above is of great medical importance in the development of the effectiveness of anti-bacterial drugs causing these diseases in humans [2-8], [3-7]. Therefore, the present study aimed to:

- Investigation of the presence of urease enzyme for some plant sources of Leguminosae.
- Study the effect of some factors affecting the extraction of enzymes from the selected plant source.
- Enzyme purification using available chromatographic methods such as ion exchange Chromatography and gel filtration Size Exclusion Chromatography.

2. MATERIALS AND METHODS

The current study included the selection of a number of seeds of the legume family plants for the purpose of obtaining the urease enzyme. The most efficient plant is selected in terms of enzyme efficiency. The enzyme is

then extracted, purified and labeled and the optimal conditions for urease efficacy are studied.

2.1 Collection of samples

The Iraqi seeds belonging to the legume family plants were obtained from the local market and included(*Vigna unguiculata, Vicia faba, phaseolus vulgaris and Lagonychium farctum*), The seeds were selected for sound testing.

2.2 Seed preparation

The seeds were placed in the Petri dishes containing the filtration papers moistened with distilled water at a temperature of (32c0) until reaching germination stage to obtain the highest efficiency of the enzyme at this stage [2].

2.3 Extract of urease enzyme

The enzyme was extracted according to the method of [4] . The developing seeds were weighed at 50gm and tested with 100ml of Potassium phosphate buffer (PH = 8) and 50mM containing 10mM of ß-mercaptoethanol, Collect the ground and spray into four layers of gauze and centrifuge centrally (11000rpm) for 30min.

2.4 Measure of Enzyme Activity

The method mentioned was followed by [4],in measuring the efficacy of the enzyme on converting one micromol from urea to ammonia.

2.5 Purification of urease enzyme

The urease enzyme (EC 3.5.1.5, urea amidohydrolase) was extracted and extracted from *Lagoonchium farctum L*. Different purification methods were used. Sedimentation with ammonium sulphate and ion exchange was carried out using the DEAE -Cellulose column and gel filtration using the Sephadex G-200 column.

2.6 Characterization of partially purified urease enzyme

• Molecular Weight

Electrophoresis was used in the polyacrylamide gel to identify the molecular weight of the purified urease enzyme according to the method described by Laemmli (1970).

• Optimized pH designation for urease efficacy

The efficacy of the enzyme in the solution with the different values of the different pH numbers was estimated in the same manner as in the enzymatic efficacy evaluation.

• The optimal temperature for enzyme activity

Enzyme efficacy was estimated at temperatures ranging from 20-50 m

3. RESULTS AND DISCUSSION

3.1 Detection of Urease Enzyme in Seeds of some legume Family Plants

The enzyme Urease was investigated in a number of legume family plants by detecting the highest enzyme activity in those sources to select the appropriate source of enzyme production. The results indicated in Table (1) indicate that there is a difference in the effectiveness of the urease enzyme by different sources. Lagonychium farctum is the best plant source for the legume family in its content of urease enzyme, which has an enzyme activity of 2.613 units/ml.

Table 1: The enzymatic and quality activity of some seeds of the legume family plants.

Seeds	Vol	Enzyme activity	Protein. Cons	Specific activity
Lagonychium farctum	50	2.613	10.654	0.250
Vicia faba	50	1.53	12.152	0.126
phaseolus vulgaris	50	1.583	12.613	0.126
Vigna unguiculata	50	2.165	8.889	0.244

3.2 Purification of the urease enzyme extracted from the seeds of the *Lagonychium farctum* plant

Urease was purified from the *Lagonychium farctum* plant extract using various chromatography techniques including ion exchange chromatography and gel filtration after deposition with ammonium sulphate, with varying precipitation rates (75%, 50%, 25%) and showing The results shown in Figure (1) showed that the best enzymatic efficacy was at saturation rate (50%) as it reached (15.29) unit/ml compared to raw extract.

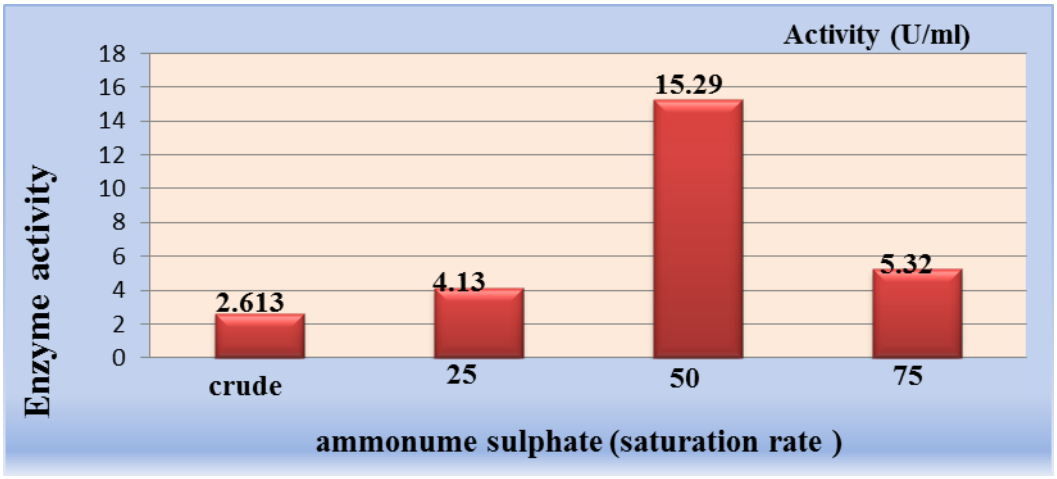

Figure 2: Determination of the optimum saturation ratio of ammonium sulphate in the concentration of urease enzyme extracted from plant seeds *Lagonychium farctum*

3.3 Purification using ion exchange

The ionis extracted from the *Lagonychium farctum* seeds was purified with Ion Exchange Chromatography using the DEAE-Cellulose column. This technology has been used for its high capacity for bio-separation, high capacity to bind proteins in addition to easy to accomplish. The results indicated in Figure 2 showed that the highest enzymatic efficacy and efficacy of urease was 8.98, 3.5, respectively, with an enzyme yield of 54.98%. Some studies have indicated the use of an ion exchanger DEAE-Cellulose in the purified urease of Chenopodium albumen, where the specific efficacy was 1.9 units / mg and 12% enzymatic yield [5], [9-13].

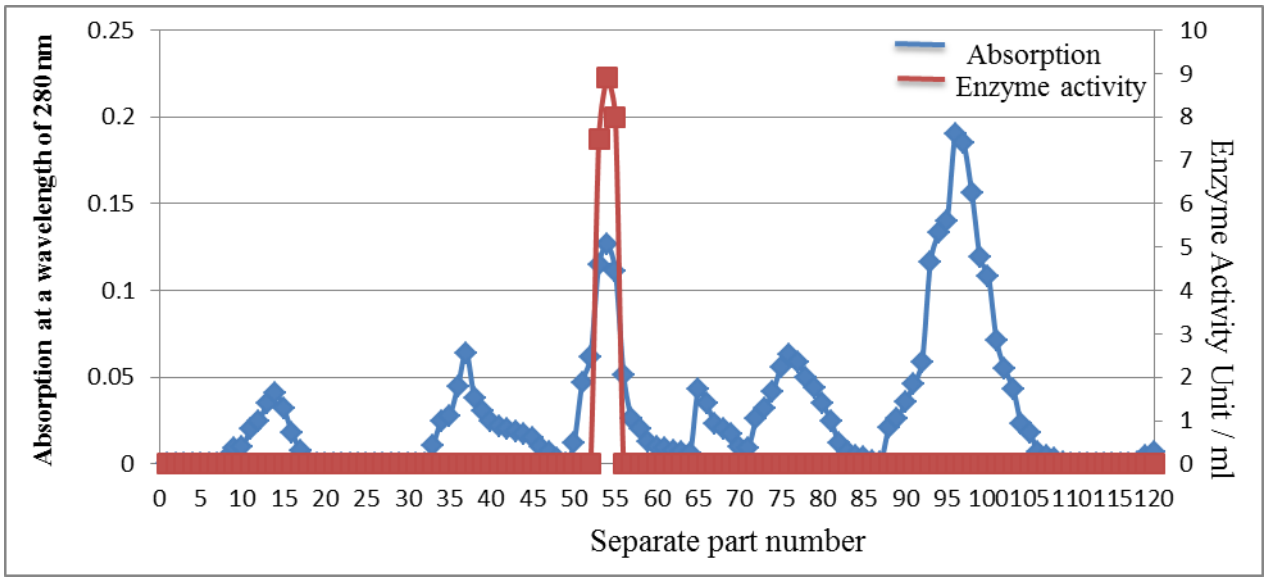

Figure 2: Ion exchange chromatography to purify the urease enzyme using the ion exchanger DEAE -Cellulose with a distance of 1.25 x18 cm

3.4 Purification using gel filtration chromatography

The next step was to purify the urease from the *Lagonychium farctum* seeds after the ion exchange step, loaded 10 mL of partially purified enzyme to the Sephadex G-200 column with dimensions of 32 x 1 cm, previously balanced with solution potassium phosphate. The Sephadex G-200 has separation limits within the range (5000-600000) Dalton, which allows the ability to separate with a high degree of purity [11-14]. The proteins eluted during the column at a flow rate 1 ml / min and collected at 5 ml / fraction. Protein peaks were detected by measuring the optical density at 280nm wavelength using ultraviolet light.

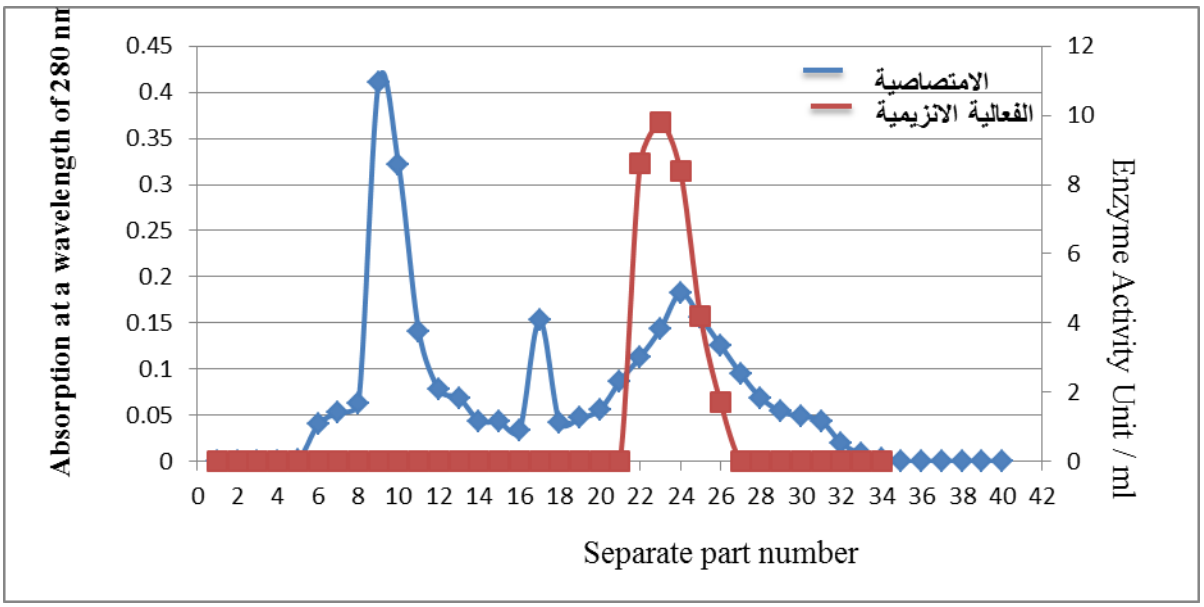

Figure 3: Chromatography of gelatinous filtration of urease extract from Kharnub plant using G-200 sulfide gel column with dimensions (32X1) cm

3.5 Determination of molecular weight using SDS-PAGE

We used the method described by Laemmli(1970) in estimating the molecular weight of purified urease from the seeds of the *Lagonychium farctum* plant, The results show with molecular weight of the urease enzyme is approximately 85 kDa. The diffrent molecular weight of the urease because of the variation in the number of constituents of the enzyme.

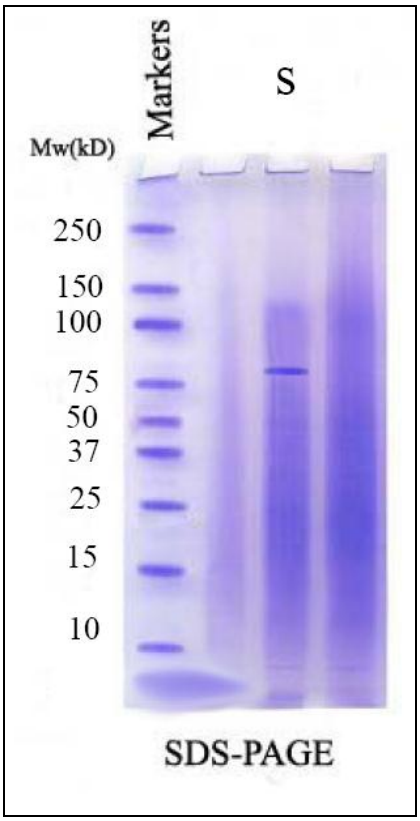

Figure 4: Electrical transfer of purified urease from *Lagonychium farctum* seeds on SDS-PAGE gel

- **Mw(KD):** Molecular weight (**kilo Dalton**).
- **S:** Sample (**Urease enzyme**).
- **Markers:** Standard protein **ladder**.
- **SDS-PAGE:** Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

The optimum pH of urea was determined by incubating the purified urease solution with the urea at different values of pH (4). The results indicated in Figure 3-9 show that purified urease gave the highest enzymatic efficacy at pH 8.0, with an efficiency of 8.6 units / mL.

3.6 Determine the optimum temperature of the urease

The optimal temperature of urea was determined from the use of different temperatures ranging from (20-50) m for 15 minutes. The results showed that the optimum temperature of the urease was 37 ° C. The enzymatic efficiency was increased by increasing the temperature to reach a maximum of 37 ° C

Some studies have indicated that purified urease from *Yersinia enterocolitica* has the highest enzymatic efficacy at an optimal temperature of 65 ° C [2-6], [15]

4. CONCLUSION

1. The legume family is efficient in its content of urease and can be considered one of the most important sources of the enzyme.
2. The enzyme isolated from the chickpeas works at a pH of 8.0 in the Potassium phosphate solution.
3. The use of traditional purification steps is essential in purifying the urease enzyme from the seeds of the plant.

The molecular weight of the partially purified urease enzyme from the chickpea seed is 85 kd

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