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RESEARCH ARTICLE

Urease Activity Level in Crude Extract From Peels of Some Legumes and Cucurbits

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Abstract

Ureases are an important group of enzymes with various applications in the analysis of food and water, chemicals, clinical, agricultural and pharmaceutical industry, besides having great interest for the treatment the industrial waste. The main objective of the present project is to extract urease enzyme from economical sources which can be utilized in biotechnology, medicine and industrial fields, as well as clean the environment from the plant wastes and recycle it. In this study urease was extracted from peels of some legumes and cucurbits: (bean, pea, water melon and melon), as well as bean seeds were also used as a known main source of urease for comparison. The enzyme was extracted from peels by electrical blender homogenization with phosphate buffer (0.2 M, pH 7), then protein concentration, urease activity, pH stability, thermal stability and storage stability were determined in crude extract. Urease activity and specific activity extracted from pea peels was the highest among the other extracted wastes, which showed stability at neutral pH 7 at room temperature, also it was very stable when incubated at (40-50 °C) for 20 min, and during storage at -18 °C at pH 7 for 3 months indicating that this enzyme was very stable. No significant differences were found between urease activity using pea peels with that measured by standard kit for the blood urea determination in serum of normal and patients. So, pea peels considered as an important economic source of urease enzyme.

Keywords: *Urease activity, Pea peels, PH stability, Thermal stability.*

Introduction

Urease, a nickel-dependent metalloenzyme (EC 3.5.1.5, urea amidolayse) [1], is one of known highly efficient enzyme that belongs to amidohydrolase and phosphotriesterase [2], that carrying out the rapid catalysis and hydrolysis of one molecule of urea to yield two molecules of ammonia and one molecule of carbon dioxide [3-6]. Urease is found in many organisms including several higher plants, some bacteria, fungi, yeasts, algae and invertebrates [7-8].

It has been shown that urease play an important role to recycle nitrogen bound in urea that accumulates during early seedling development [9]. In 1926, Sumner was the first chemist who showed that urease was a protein of the globulin type with an isoelectric point of five [10], the best biochemical characterized plant urease from jack bean (*Canavalia Ensiformis*), was the first enzyme to be crystallized [11-13]. Plant and fungal ureases are homo-hexamers of 90 KD subunits. On the other hand, bacterial ureases are multimers of two or three subunit complexes. The plant, bacterial and fungal ureases have the similar sequence and have highly conserved secondary structure and the same catalytic mechanism [5, 6]. Several studies were carried out to isolate and purify urease enzyme using different biochemical techniques from different plants such as water melon seeds, mul berry leaves, bean seeds, zahdi dates palm seeds, chickpea seeds, pea seeds, and asparagus bean [14-20].

Some of urease's applications are used in clinical diagnostic kits for determination of blood urea, haemodialysis systems, treatment hypertension, antibiotic production, vaccines, and wine industry as urea reducing agent [21-23]. The study aimed to extract urease enzyme from peels of some legumes and cucurbits as cheap sources, as well as using simple extraction method, rapid, and an inexpensive that included single step extraction by homogenizing the peels by electrical blender with phosphate buffer only.

Materials and Methods

Samples

Fresh and mature legumes and cucurbits were purchased from the local market in Baghdad city. All laboratory chemicals and reagents throughout this study were of highly purified grade.

Extraction of Urease

The enzyme was extracted by homogenizing 25 gm of peels with 75ml cold potassium phosphate buffer (0.2 M, pH 7) in electrical blender for 5 min, the homogenate was filtered through cheese cloths and was centrifuged at 3000 g for 5 min. The supernatant was regarded as crude enzyme [17].

Urease Activity Assay

Urease activity was assayed according method described by (Mobley *et al,* 1987) [24], which is based on using urea as substrate, urease catalyzed the hydrolysis of urea and liberated ammonia, which reacts with hypochlorite to form a monochloramine which then react with phenol to form blue-complex whose absorbance is measured at 625 nm. One unit of urease activity was defined as the amount required for liberating 1 µmol of ammonia per minute at 37ºC.

Determination of Protein Concentration

Protein concentration was measured using Bradford method [25]. The used standard protein was bovine serum albumin (BSA).

PH Stability

To investigate the effect of pH on enzyme stability, the crude enzyme was incubated at room temperature for 30 min in buffers of various PHS (5.5-8), and then residual urease activity assay was determined.

Thermal Stability

To study the effect of temperature on urease stability, the enzyme solution was incubated in water bath at 40,50,60,70 and 80 ºC for 30 min. The residual urease activity was measured at 37ºC temperature.

Storage Stability

To study the effect of storage on enzyme stability, the enzyme solution was stored at -18ºC for 3 months, and the activity was de ermined every 10 days.

Urease Applications

Determination of Blood Urea

Urea was determined in sera samples of normal and patients with urea disorder in two ways; the standard kit (linear chemicals company) and the crude urease extracted from pea peels.

Statistical Analysis

All parameters were measured in triplicates and the results were represented with standard deviation calculated by Microsoft excel program. The differences between groups were tested by t-test, and p value was considered significant if it is < 0.05.

Results and Discussion

Bean peels, pea peels, water melon peels, melon peels, and bean seeds were screened for protein concentration, urease activity and specific activity to select the best urease source among peels. Table (1) shows that the highest urease activity and its specific activity possessed by pea peels (362.6 ± 4.16 U/ml and 149 ± 2 U/mg) respectively, while the lowest urease activity possessed by melon peels (44 ± 3.6 U/ml).

Table 1: Mean values ± SD of protein concentration, urease activity and specific activity

Samples	Protein concentration (mg/ml)	Urease activity (U/ml)	Specific urease activity (U/mg)
Bean peels	1.16 ± 0.05	84 ± 3.60	71.66 ± 6.65
Pea peels	2.43 ± 0.07	362.6 ± 4.16	149 ± 2
Water melon peels	0.38 ± 0.05	44 ± 3.6	115 ± 5.77
Melon peels	1.73 ± 0.05	232 ± 7.21	130 ± 5
Bean seeds	8.36 ± 0.15	323.33 ± 7.63	38.6 ± 0.52

In comparison with other studies, Ahmed and Bedewia, 2005 extracted the urease enzyme from *Vicia Faba L* (Bean seeds) by homogenaization it with cold distilled water by electrical blender, they obtained a specific activity of (94.08 U/mg) [16]. Al-shikrichy *et al*, 2010 obtained crude urease enzyme from extract of *Phoenix Dactyli Fera. L.* (Zahdi dates palm seeds) by mixing the seeds powder with phosphate buffer, the specific

activity of extracted urease was (629.70 U/mg) [17]. El-hefnowy *et al*, 2014 extracted urease enzyme from germinated *Pisum Sativum* (pea seeds) pasted in a mortar and pestle and then suspended in 20% chilled acetone -20 ºC, the specific activity of urease extracted equal to (454.5 U/mg) [19]. The crude urease of the pea peels was kept at pH range (5.5-8) for 30 minutes using suitable buffer solution.

The residual activity was assayed at the optimum pH. The result illustrated in Fig (1) showed that maximum stability for pea peels extracted urease was obtained when pH was adjusted to 7. The pH-activity profile of pea peels urease was typical bell-shaped curve. In PHS ranging (5.5-6.8); urease lose only (20% -35%) of its total activity, while in higher PHS more than 7.5 it lose 35% of its total activity.

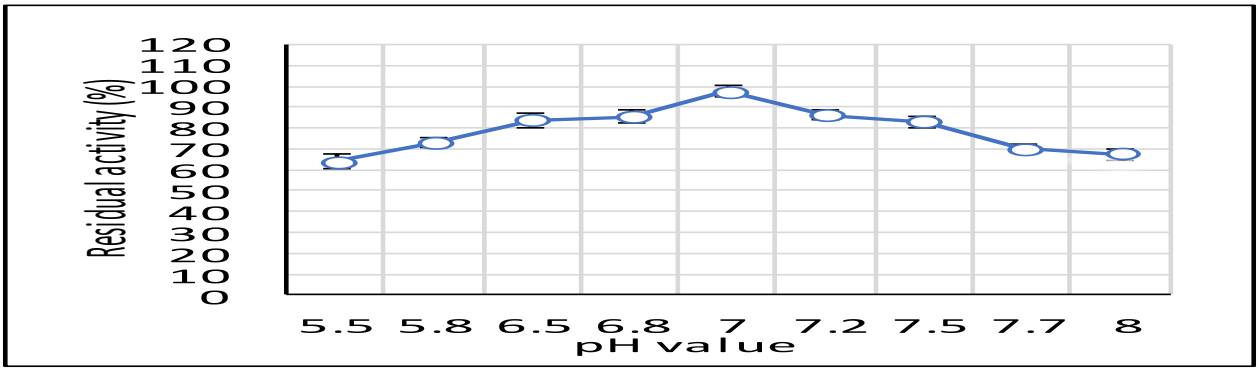

Fig 1: Effect of pH on urease stability, the vertical bars represents standard deviation

In general, most plants show maximum enzyme activity at or near neutral pH [26], it has been reported that most plant ureases have its maximum activity in pH of (6.8-8.3) [16-19]. The pH stability of an enzyme is an important for the spesify of the conditions that should be exist through isolation, purification, handling and storage of the enzyme, this stability is affected by some factors such as, source, type and purity of the enzyme [27]. The effect of pH on the enzymatic activity and stability could be explained by causing it to become irreversibly inactivated by changing the state of ionization in the active center of the enzyme, one of the ionized form of the protein is only active, so that a change in pH at either side of the optimum produces a decrease of this form and hence a fall in the activity [28]. It is obvious from the result illustrated in Fig (2) that urease extracted from pea peels was stable, it retained 100% of its original activity after 30 minutes of incubation at 40ºC and 50 ºC, while retained about (81%, 71% and 58%) at (60 ºC, 70 ºC and 80 ºC) respectively. Many researchers study the effect of the temperature on urease activity, these results agreed with those of mohammed *et al*, 1999 who showed that urease purified from water melon seeds at pH 7.5 did not loss enzyme activity recorded up to 40ºC for 30 min [14].

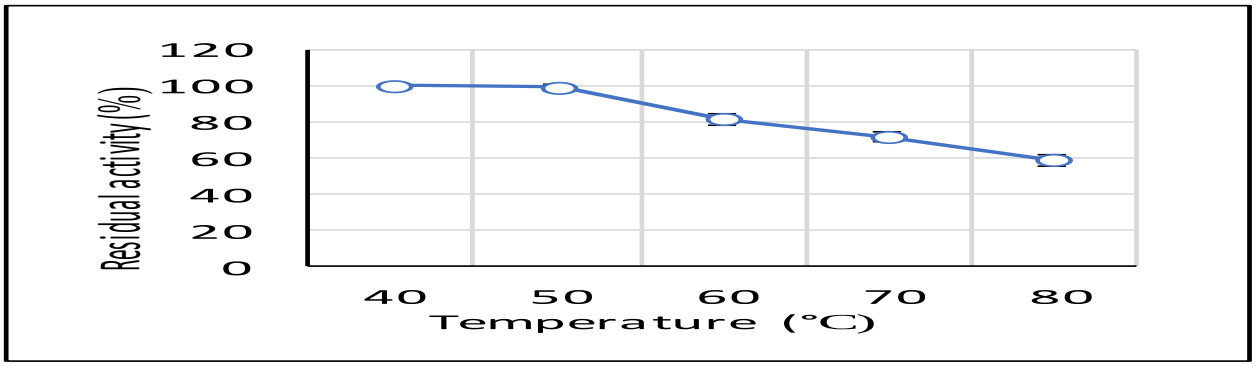

Fig 2: Effect of temperature on urease stability

The catalytic activity of enzymes results from a precise and highly ordered tertiary structure. The tertiary structure of an enzyme is maintained primarily by a large number of weak non-covalent bonds. That can be affected by temperature. A high temperature will increase the enzyme and substrate collision and this is offset by the

increasing rate of the denaturation [30]. Urease solutions were stored at -18 ºC and the residual activity was measured each 10 days to determine the loss activity during period. Result in Fig (3) indicated that urease extracted from pea peels was most stable in freezing storage; it retained 100% of its original activity; at the first month, and more than 95%, 88 % of its original activity at second and third month respectively. These results agreed with those of Ahmed and Bedewia, 2005 they reported that urease retained 70% of its original activity when stored at 4 ºC for 30 days [16].

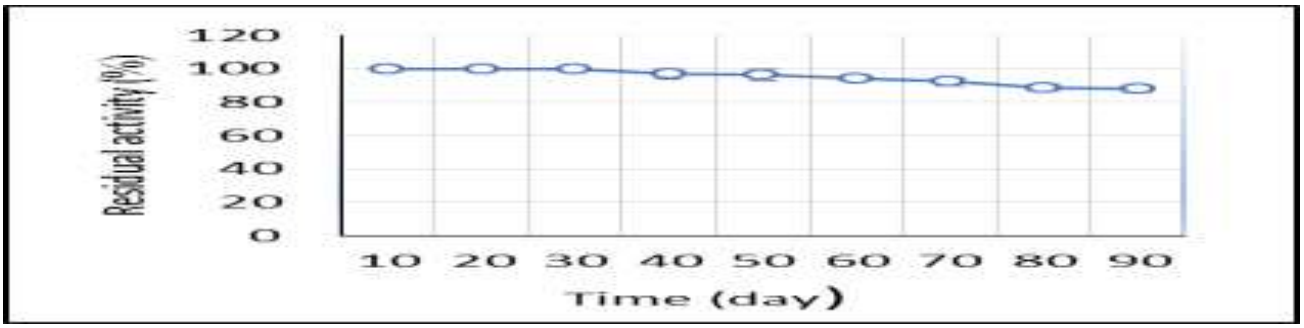

Fig 3: Effect of storage on urease stability

The results illustrated in Table (2) indicated that the crude extracted urease from pea peels gave comparable activity to that urease available urea standard kit for determining blood urea. This agreed with previous studies [14, 16].

Table 2: mean values of blood urea concentration in both normal group and patients group

Group	Sample number	Blood urea concentration (mg/dl) by using from pea peels extracted urease		Blood urea concentration (mg/dl) by using urea standard kit	
		mean± S.D	Range	mean± S.D	Range
Normal group	20	32.6.45± 8.78	25-40	34± 6.73	26-42
Patients group	20	78.75± 8.53	70-90	79.75± 8.99	73-93

Conclusion

Pea peels can be considered one of the economic sources of urease enzyme as important, as bean seeds, which can be utilized in different fields of medicine, technology, and industry.

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