Purification and Charcterization of Pectinesterase from Potato

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Abstract

Pectin esterase was extracted from potato and determining its activity were investigated. Crude enzyme recorded specific activity 25.18 U / mg and increasing specific activity of the enzyme after dialysis concetraion recording 107.14 U/ mg, then the enzyme peak after using anion ion-exchange chromatography was recorded a specific activity reached 192.31 U /mg and fold of the purification 8.2 and yield 23.34%, either gel filtration was the fold of the purification 19.8times and with specific activity 476. 28 U / mg with the proceeds of 50. 68%. The optimum pH of the pure enzyme was 7.0 and optimum temperature was 50°C, while the stability of the pH of the pure enzyme was pH 8.0 and temperature stability at 60°C.

Keywords: Extraction, purification, properties, pectinesterase, potato.

Introduction

Pectinesterase (pectinesterase, PE, E.C. 3.1.1.11) is widely distributed in microorganisms and plants. In plants, PE is bound to the cell wall by electrostatic interaction. It catalyzes the de-esterification of esters of polygalacturonic acid polymers to form methanol and pectinic acids/ pectic acids [1, 2]. PE has been purified and characterized from several fruit sources, including orange [3], tomato [4]. From other sources of plant, PE has been extracted and partially purified from potato [5] and from seeds of Ficus awkeotsang [6,7]. Some studies established that plants contain different forms of PE [8,9]. Control of PE activity in situ is very important in the food industry because of its influence on the final product quality; particularly to produce low methoxyl pectins in citrus peels [10] to obtain turbid citrus juice [11] and high viscosity tomato juice and puree [12] to improve texture and firmness in some processed fruits and vegetables [13,14] and color, limpness and other physicochemical parameters of fried potatoes [15,16]. For this reason, the extraction, quantification, purification and characterization is needed if one wishes to measure the effect of temperature on activation/ inactivation processes during the Low Temperature-Long Time (LT-LT) blanching and to design the better blanching conditions [17].

Materials and Methods

Potato tubers were purchased from a local market, Baghdad. Sodium chloride was used for enzyme extraction and some other analytical grade chemicals were obtained from Sigma.

Extraction of pectin esterase

PE from potato tubers was extracted by mixing (g) of potato with (ml) of (1.92M) NaCl, blendind for 15 min and centrifugate the mixture at 6300 rpm/30 min/4°C finally the supernatant was represented as a crude enzyme.

PE Assay

PE activity was assayed by the titration method proposed by [18]. This method contains the measurement of the releasing rate of carboxyl groups in a pectin solution (1% w/v), pH of 7.0 at 30°C. The substrate was prepared and stored according to the procedure described by [19].

The initial rate or reaction was obtained when the free carboxyl groups were titred with 20 mM NaOH, considering that the equivalent amount of NaOH solution used is proportional to the PE activity. One PE activity was defined as the amount of the enzyme able to release 1 mol of carboxyl groups per minute under the above mentioned reaction conditions.

Activity (U/ml) = (ml of NaoH)*(molarity of NaoH)*(1000/Time)*(ml of enzyme)

Protein determination

The concentration of protein was determined according to the Bradford method, using a standard curve of bovine serum albumin.

Pretreatment of the PE extract crude

PE crude extract obtained from potato suspension was dialyzed in cellulose membrane against a phosphate buffer 0.1 M (pH 7.0) during overnight for 12 h at 4°C.

Pre-purification of PE

Dialyzed and microfiltrated PE extract was used to carry out a chromatography.

Anion exchange chromatography

The ion exchange chromatography was used to performed an anion exchange chromatography using a column DEAE-Celullose (3×31) cm The concentrated samples were applied to a column of anion exchange, equilibrated with 0.02 M sodium phosphate buffer (pH 7.5) containing 0.3 M NaCl and 0.02% sodium azide.

Gel filtration chromatography

PE was purified by gel filtration chromatography using a column (2×75 cm) packed with Sephacryl S-300 and equilibrated with 0.1 M sodium phosphate, (pH 7.0) Elution of the protein was carried out at 22 mL/h flow rate with the equilibrating buffer. Fractions of 4.6 mL were collected

Optimum pH and thermal stability of PE

The pH dependence of potato enzyme was evaluated at a pH ranging 3.0-9.0 at 25°C, using the titrimetric method of [18].

Optimum temperature and thermal stability of PE

Temperature dependence of potato PE was evaluated at a temperature ranging 30 – 90°C at optimum pH, Each sample of PE (1.0 mL) was preheated for 30 min at each temperature tested and immediately the PE activity was assayed.

Results and Discussion

Purification steps are shown in Table (1). The procedure of PE purification was achieved with a protocol consisting of four steps. The first step gave a 100% yield. This value was higher to the values obtained for Ficus awkeotsang PE [20], which were 75%. On the other hand, this value was approximately equal to the values obtained for tomato PE [21] and mandarin orange PE (96%) reported by [22]. many proteins presents in the dialyzed extract, giving one active fraction. These results were similar to those reported to PE from mandarin orange fruit by [22]. During the third step, the possibility of three enzymatic forms was rejected due to that one active fraction was obtained. In this step, the enzyme was found to be eluted as a single peak. After this step, a 23.34% yield was reached and the PE was purified 8.2 folds with specific activity 192.31 (U/ml). For further purification of the potato PE, a gel filtration chromatography was used. The purified PE gave specific activity 476.28 (U/ml), a 50. 68% yield and 19.8 fold purification. This step was the major feature of the protocol proposed due it showed a good selectivity for PE, in fact, by this step the purification factor was higher.

Table (1)
Stepe of Purification of pectin esterase extracted from potato.

Steps	*Volume (ml)*	*activity (U/ml)*	*Protein (mg/ml)*	*Specific activity (U/mg)*	*Total activity (U)*	*Purificati --on fold*	*Yield%*
Crude extract	123	10.45	0.415	25.18	1285.35	1	100
Dialyzed	35	22.5	0.21	107.14	787.5	4.25	61.27
Anion exchange	12	25	0.13	192.31	300	8.2	23.34
Gel filtration	16	40	0.084	476.28	640	19.8	50.68

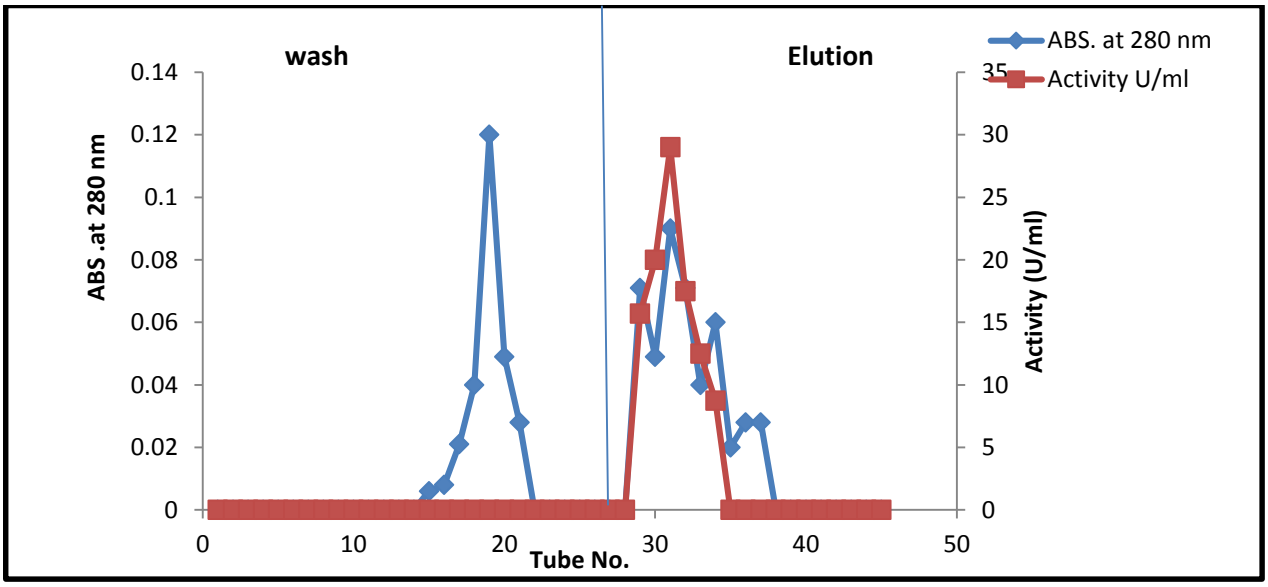

Fig.(1): Ion exchange chromatography for polyphenol oxidase extracted from broccoli stems DEAE-cellulose column (3 X 31 cm) equilibrated and washed with exchange, equilibrated with 0.02 M sodium phosphate buffer (pH 7.5) containing 0.3 M NaCl and 0.02% sodium azide. at a flow rate (6.5 ml/4.5 min).

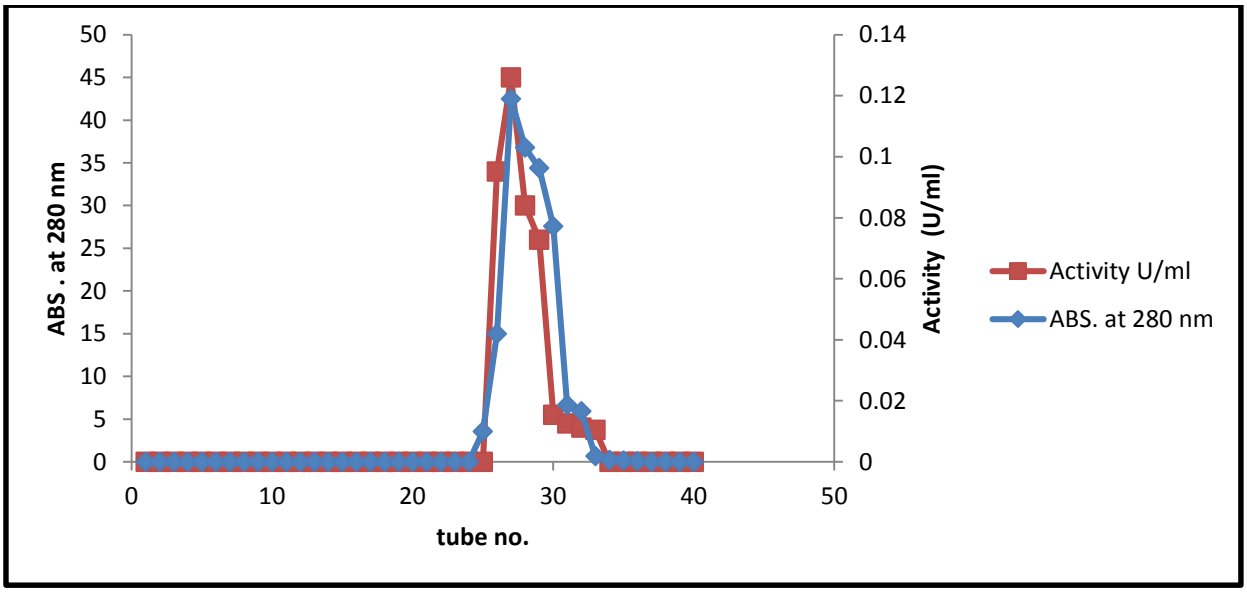

Fig.(2): Gel filtration (2 × 75 cm) column packed with Sephacryl S-300 and equilibrated with 0.1 M sodium phosphate, (pH 7.0) Elution of the protein was carried out at 22 mL/h flow rate with the equilibrating buffer. Fractions of 4.6 mL were collected.

The thermal stability of PE Fig.(3) was calculated by incubing the enzyme for 30 min at increasing temperature. The activity was substantially increased up to 60°C and then it decreased to about 10 U/ml at 70°C and 4.99 U/ml at 90°C. The PE from potato alpha cultivar appeared thermostable. The thermal stability of this enzyme was considerably higher than that found for other PEs, which generally was up to 60°C. These results are not similar to those reported by [5], who obtained an optimum temperature of 55°C and a Q10 of 1.33 in the temperature range of 15 to 45°C. It is important to note, that the PE from potato Alpha Cultivar was highly stable in a wide temperature range (30 up to 90°C), which could be very attractive for the thermal processing of this cultivar. Last point is very important because, LT- LT blanching process is generally carried out at temperature range between 50–70°C with the objective of an in situ activation of native PE. This study demonstrates that the maximum activity of potato PE is around 60°C, and this temperature is the optimum for reach better textures in potato tissue by LT-LT blanching.

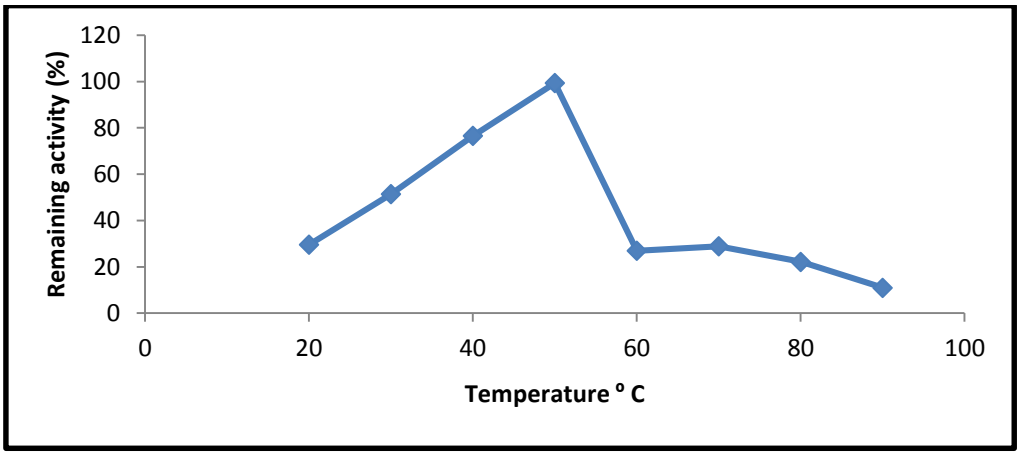

Fig.(3): Thermal stability for pectin esterase purified from potato.

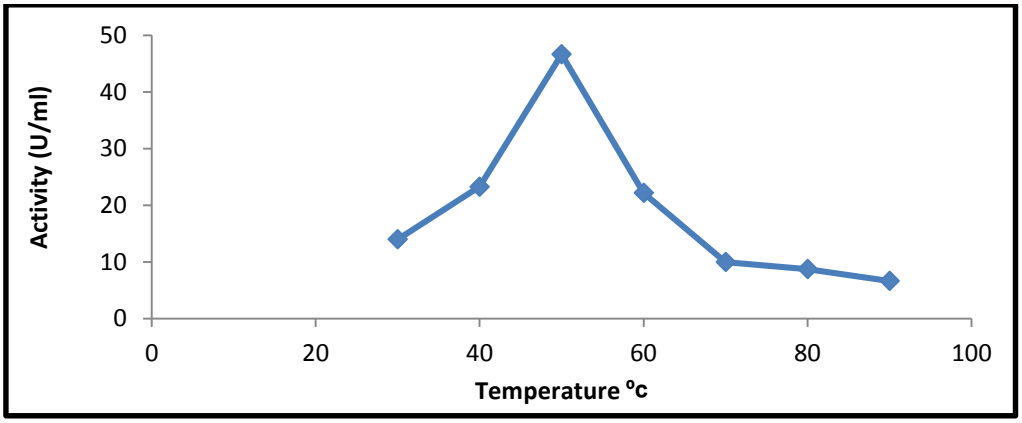

Fig.(4): optimum temperature for pectin esterase purified from potato.

The effect of pH on PE activity is shown in Fig.(3). The PE enzyme showed a maximum activity at pH 8 and was under detectable below pH 5.0. The pH optimum found for potato PE was similar to that found for PEs from fruit sources, which generally was in the range 7-9.

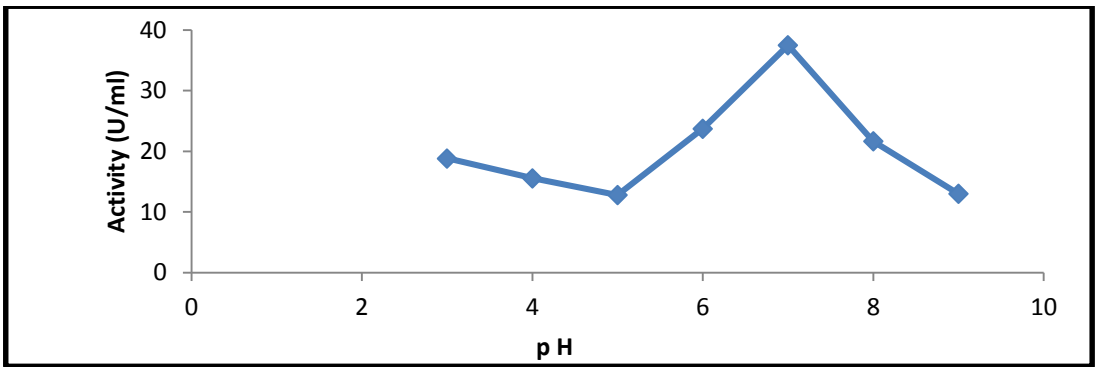

Fig.(5): Effect of pH on activity of pectin esterase purified from potato.

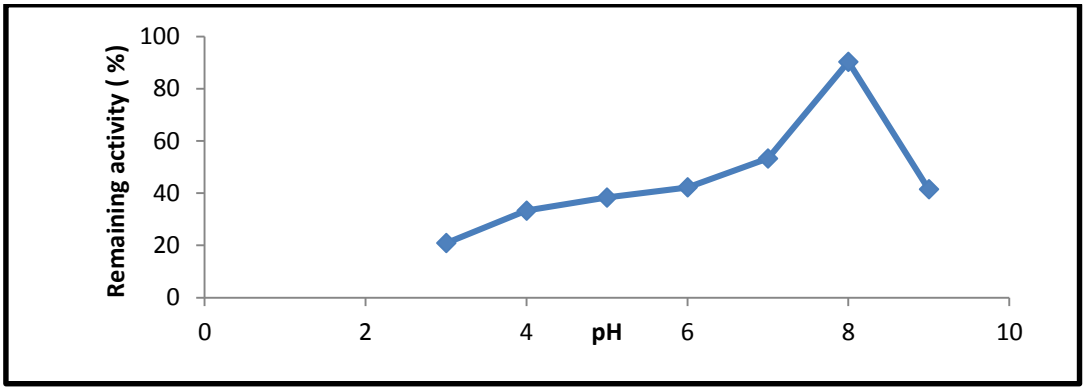

Fig.(6): Effect of pHon stability of pectin esterase purified from potato.

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الخلاصة

استخلص البكتين استريز من البطاطا وقدرت الفعالية وتركيز البروتين، حيث سجل الإنزيم الخام فعالية نوعية مقدارها ٢٥,١٨ وحدة/ ملغم وسجلت زيادة في الفعالية النوعية بعد التركيز بالديلزة وصلت ١٠٧,١٤ وحدة/ ملغم. نقي الانزيم باستخدام التبادل الأيوني حيث سجلت فعالية نوعية 192.31 وحدة/ ملغ و كان عدد مرات التنقية 8.2 مع حصيلة 23.34٪. أما الترشيح الهلامي مصاحبة لعدد مرات تنقية فقد وصل 19.8 و فعالية نوعية 28. 476 وحدة/ ملغم مع حصيلة ٥٠,٦٨٪. سجل الرقم الهيدروجيني الامثل للانزيم النقي ٧,٠ و كانت درجة الحرارة المثلى ٥٠ م°، في حين أن ثباتية الرقم الهيدروجيني للانزيم النقي pH 8.0 والثباتية في درجة الحرارة عند ٦٠ م°.

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