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Characterization of Pectin Methylesterase from *Aspergillus niger*

ABSTRACT

The present study was undertaken to evaluate the characteristics of pectin methylesterase produced from *Aspergillus niger*. The enzyme had a molecular weight of 59.668 kDa. The enzyme exhibited maximum activity at 45°C. The enzyme lost most of its initial activity after 20 min of incubation at 75°C. Optimal activity and stability of the enzyme occurred at pH 4, with more than 90 % of retained activity after 10 min at pH 5. Michaelis constant (K_m) was found equal to (1) mg/ml, whereas, maximum velocity (V_{max}) was (1000) µmol/min. The catalyzed reaction by pectin methylesterase had activation energy equal to 9.66 Kcal/mol. The enzyme lost 24% and 13% of its initial activity during two months of storage at4°C and -20 °C, respectively. The enzyme was activated by NaCl, KCl and $CaCl_2$ at concentration of 25 mM and inhibited by $MgSO_4$, polygalacturonic acid, methanol, IAA and EDTA.

INTRODUCTION

Pectin methylesterase (PME) pectin pectylhydrolase, (EC 3.1.1.11) is a pectinase which hydrolyze of methyl ester groups into methoxylated pectin, and methanol (Damodaran, 2007). After pectin desertification reaction by PME, low methoxylated pectin can be resulted by some pectinase as polygalacturonase. Higher plants contained large quantities of PME, whereas, some microorganisms (fungi, bacteria, yeasts) produced PME as an extracellular component (Alkorta *et al*., 1998: Aehle, 2004). It can work on pectic components causing many changes during ripening; harvest; storage and processing of fruits and vegetables. Some enzymes like PME have ability to analyze pectic substances, therefore, could be used in Food processing steps for vegetable and fruit.

Nowadays, interest in liquid enzymes has developed as they are associated with many benefits. They are nonpolluting therefore; safe, easy to use. Moreover, enzymes go into process medium instantly; disperse evenly through the application process, unlike; to powder products. Fungal PME used in the food processing because fungi are important producers of pectinase and their optimum pH is more relevant for pH of food juices, which range from (3.0 to 5.5) (Simpson, 2012).

In recent years, PME is used as processing aids in fruit and vegetable processing, although this require reducing the PME activity to stabilize the final product. Commercially, heating is commonly applied to avoid undesirable effects in food products, and to eliminate residual PME after achieving the required amount of changes when PME is used. This is especially significant during the processing of high PME foods (e.g. tomato and citrus).

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Hence, when a pulpy and viscous juice is required for tomato processing, PME must be rapidly heat inactivated (hot break juices) to avoid the formation of thin product.

Considering the increasing of industrial application of PME, the present research investigated to study some properties of PME from *A. niger*.

MATERIALS AND METHODS

Source of enzyme and chemicals: Pectin methylesterase enzyme produce from *A. niger* was purchased from DSM Food Specialties (Delft, Netherlands). Other reagents used in study were of reagent grade and were obtained from Sigma (St. Louis Mo. U. S. A.).

Assay of pectin methylesterase activity: Thirty milliliters of 0.35% apple pectin in 0.15 M NaCl (pH 4.0) was incubated with 0.1 ml of enzyme in 50 ml test tubes at 45°C for 10 minutes. The reaction was stopped by keeping the test tubes in boiling water bath for 10 minutes. The contents were cooled to 45 °C and the released free carboxyl groups were determined by titrating against 0.1 N NaOH using phenolphthalein as indicator (Mandhania *et al*., 2010). Activity of pectin methylesterase was expressed as unit based on the required quantity of enzyme to give one microequivalent from carboxyl groups/ ml/ min.

Determination of molecular weight: Size exclusion chromatography (Nchienzia *et al*., 2010) with a Zorbax GF-250 column (Agilent Technologies, Santa Clara, CA) on a Shimadzu system, fitted with a SPD-20AV UV-visible detector, at a flow rate equal to 1 ml/min was used. The equilibration of column was conducted by solution of 200 mM phosphate buffer, pH 7. The eluant was monitored at 214 nm. Molecular weight markers were Carbonic anhydrase, Cytochrome C, Aprotinin and Albumin). A plot of retention time (min) versus log molecular weight was used to estimate the enzyme molecular weight.

Determination of optimum temperature: It was determined by assaying the enzyme at temperatures ranging from 20-60 at 5°C intervals.

Determination of optimum pH: The rate of pectin demethoxylation by PME was estimated in the pH range of 2.0-6.5 by adding (0.2 M) acetate buffer (pH 2.0-4.5) or (0.2 M) of phosphate buffer (pH 5.0-6.5). The concentration was 0.35% (w/v). The relative activity was then determined.

Thermal stability: The enzyme solution was held in a water bath at temperatures ranged between 45°C and 75°C before measuring the residual activity of the enzyme for various time intervals (10-30 minutes), under optimum temperature and pH for PME enzyme assay.

pH stability: Optimum pH for PME stability was determined by holding the enzyme solution at different pH values ranged from 2 to 6 for 3 interval times as (10-20-30) minutes. Acetate buffer was used in the pH range of 2-4 and phosphate buffer in the range of 5-6. At the end of incubation period, the pH values of all enzyme solutions were adjusted to 4 and their residual activities were estimated.

Michaelis constant (K_m): It was estimated by using different concentrations from pectin apple ranged between 0.5 to 5 mg/ml. K_m value was calculated by double reciprocal Lineweaver-Burk plot.

Determination of activation energy (E_a): Arrhenius plot with kelvin temperature(°K) ranging from (298-318) was done to calculate the activation energy.

Storage stability: The enzyme solution was stored at 4 °C and – 18 °C for 8 weeks. Pectin methylesterase activity was measured every week.

Determination of the effects of some cations and chemical compounds on activity: It was determined using different molar concentrations of activators and inhibitors on the enzyme activity. In this case, concentrations of (5 to 25) mM of Na^+, K^+, Ca^{2+} and Mg^{2+} were employed. The concentrations of (5 to 25) mM of polygalacturonic acid; methanol; IAA; EDTA were employed for chemical compounds.

RESULTS AND DISCUSSION

Molecular weight: The molecular weight of *A. niger* pectin methylesterase was estimated to be 59.668 kDa using size exclusion chromatography (Fig.1). This result falls within the range of molecular weight of 30-60 kDa reported for most fungal pectin methylesterase (Jaffar and Oommen, 1993).

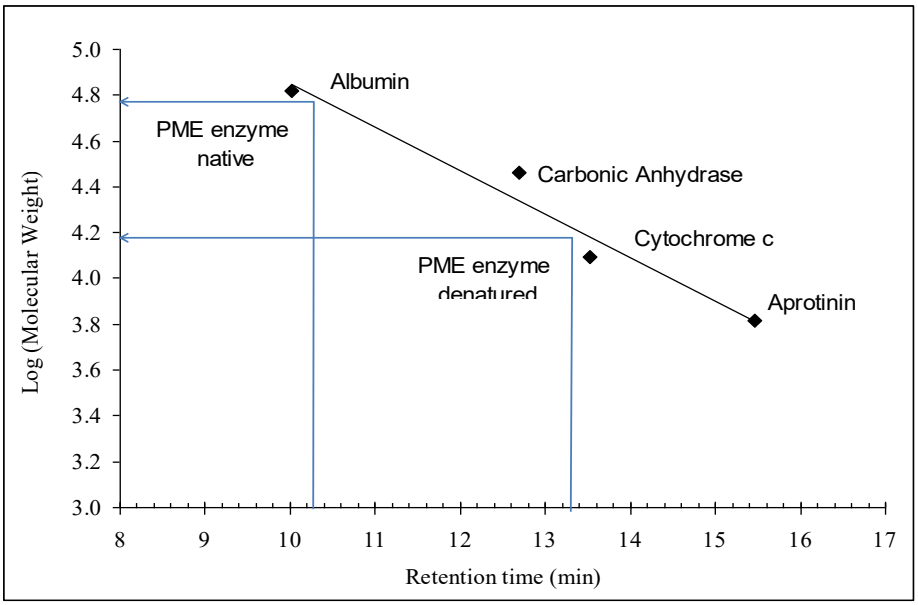

Fig (1): Standard curve for molecular weight estimation of pectin methylesterase produced by *A. niger*.

Optimum temperature of PME activity: PME activity was estimated at range between (20-60) °C. The studied enzyme showed maximum activity at 45°C (Fig.2). The obtained result was similar to that found by other investigators. (Arotupin *et al*., 2008; Christgau *et al*., 1996; Maldonado *et al*., 1994).

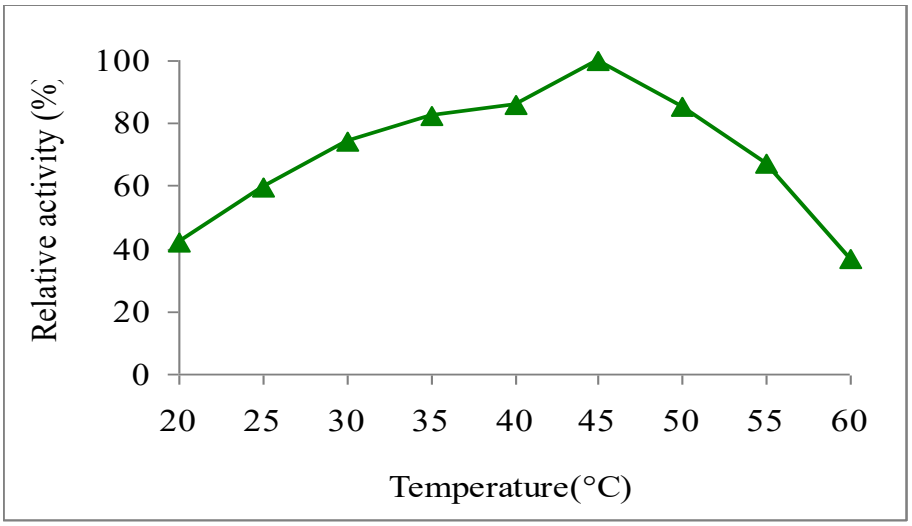

Fig (2): Optimum temperature of pectin methylesterase. Apple pectin solution (3.5) mg/mL, pH 4, 0.15 M NaCl.

Optimum pH of PME activity: The pH optimum was determined to be 4.0 at 45°C (Fig. 3). These results obtained close to the values reported for fungal PMEs which exhibit acidic range of pH optimum (4-6) (Alebeek *et al*., 2003; Huang *et al*., 2011).

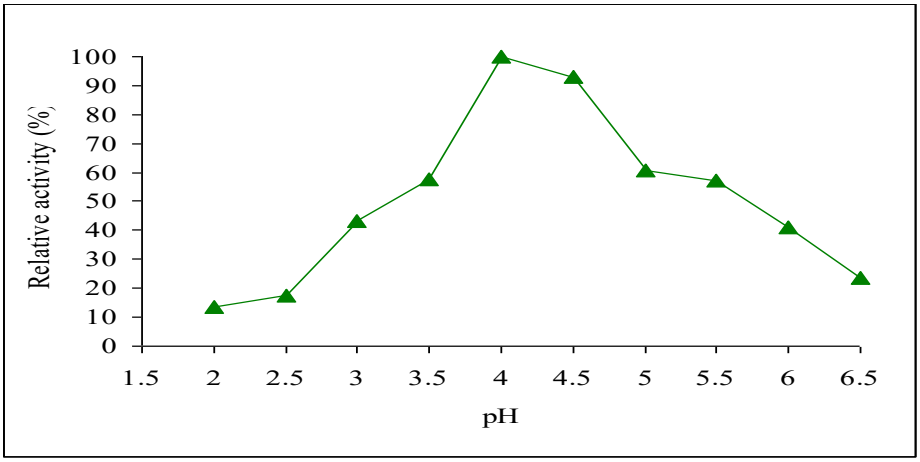

Fig (3): Optimum pH of pectin methylesterase. Apple pectin solution 3.5 mg/mL, pH 4, 0.15 M NaCl.

Thermal stability: Figure 4 shows the residual PME activity after 10, 20 and 30 minutes of incubation the enzyme at different temperatures. PME lost 77% of its original activity at 70°C and about 96% at 75°C after 10 minutes. At 75°C for 20 min the enzyme was completely inactivated. After 30 minutes of incubation at 55, 60, 65, 70 and 75°C, it lost 13, 32, 41, 89 and 100% of the original activity comparing with 18, 26, 37, 83 and 100 % after 20 min and 20, 23, 35, 77 and 96% after 10 minutes of incubation, respectively. Aizenberg *et al*., (1996) and Arbaisah *et al*., (1997) reported that PME was stable at temperatures less than 40°C. Both authors indicated that, for temperatures above 40°C, inactivation was notable after a short period of incubation.

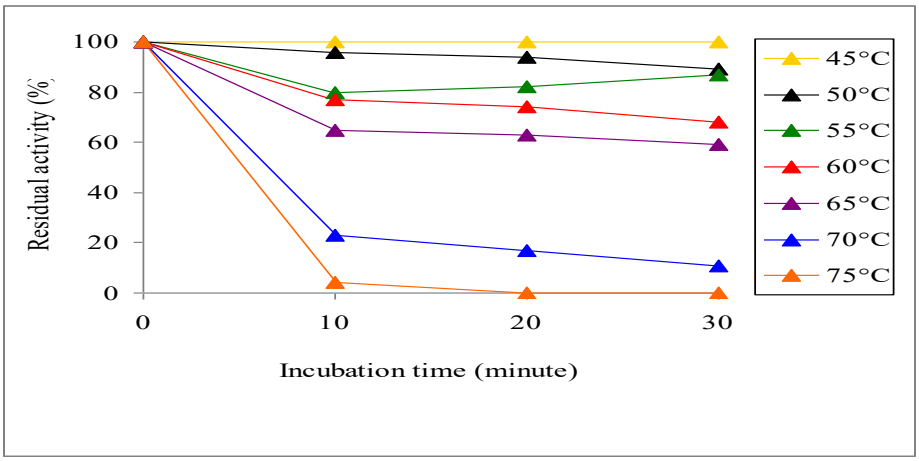

Fig (4): Thermal stability of PME. Residual activity was measured.

pH stability: The PME showed maximal stability at pH 4, with more than 90 % of retained activity after 10 min at pH 5 (Fig.5). Data was almost agreed with Aizenberg *et al*., (1996) stated that the stability of PME at pH range (4-5). However, Christgau, *et al*., (1996) mentioned that PME of *A. aculeatus* was found most stable at pH 5.5-6. Stability data showed a decline in PME activity below 4 and above 5 within pH range values under investigation. Other PMEs found stable within pH range of 5 to 8 (Javeri and Wicker, 1991; King, 1991; Jiang, *et al*., 2012).

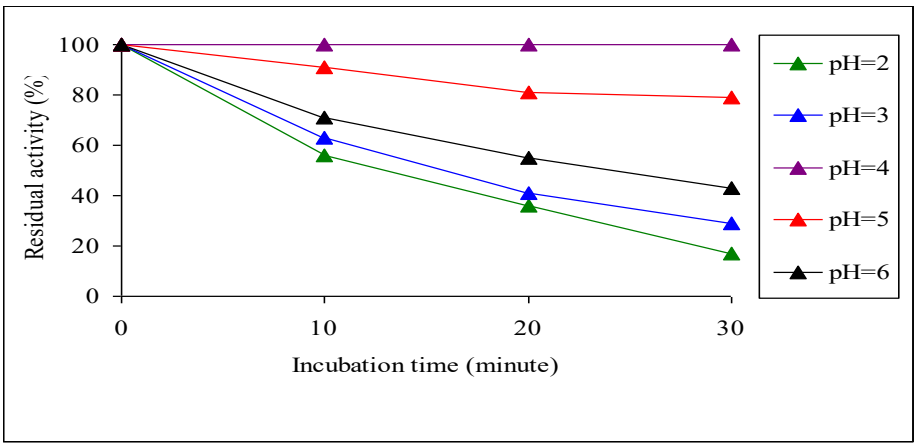

Fig (5): pH stability of PME. Residual activity was measured.

Enzyme kinetics: K_m and V_{max} values were evaluated from the graph (Fig 6). Michaelis constant (K_m) of pectin methylesterase was found to be (1 mg/ml). Maximum reaction rate (V_{max}) was 1000 µmol/min. Km refers to the concentration of substrate to fill the half active sites of an enzyme. It is also a measure of a strength of the enzyme - substrate (ES) complex . In fact, a high K_m value indicates weak binding and vice versa. Therefore, low K_m value of pectin methylesterase indicated a strong affinity toward substrate (Marangoni, 2003). K_m value obtained in the present study was very close to those of *Arthrobotrys oligospora* and *A. repens* pectin methylesterases (Jaffar and Oommen, 1993; Arotupin *et al*., 2008).

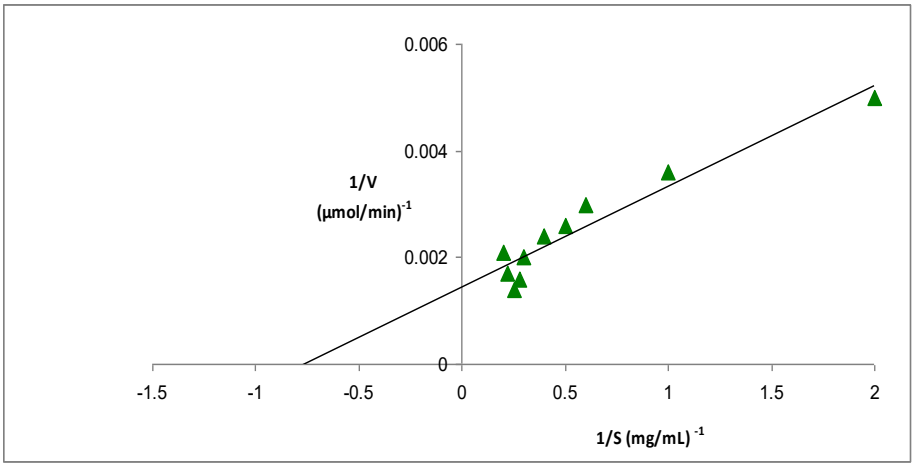

Fig (6): Lineweaver- Burk plot for estimating Km and Vmax of PME produced by *A. niger*.

The plot of Arrhenius was used to compute E_a at (298-318) °K. Energy of activation by substrate of pectin was equal to (9.66) kcal mol^{-1} (Fig.7).

This value is similar to those reported by Jaffar and Oommen (1993), for *Arthrobotrys oligospora* pectin methylesterase which estimated to be 11.9 kcal mol^{-1}. Mutlu, *et al*., (1999) and Sartoglu, *et al*., (2001), they reported activation energies of 9.36 and 9.424 kcal mol^{-1} with a commercial pectinase preparation respectively.

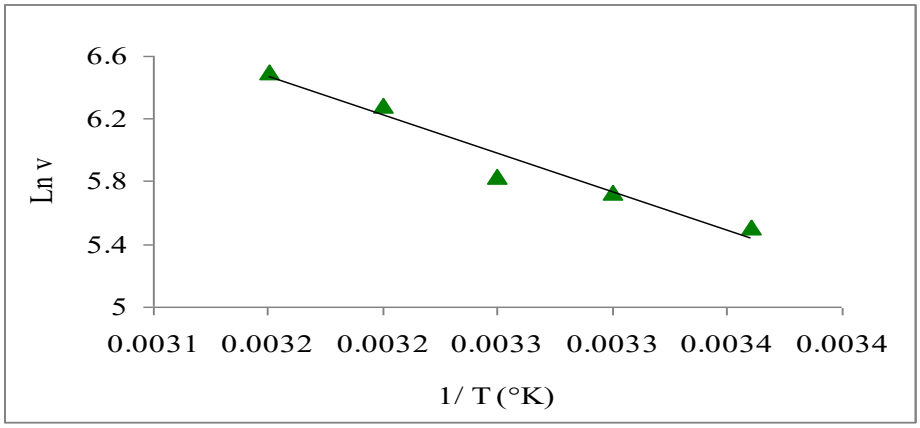

Fig (7): Arrhenius plot for estimating activation energy of PME produced by *A. niger*.

Effect of some cations and chemical compounds on enzyme activity: Concentrations of (5 to 25) mM of Na^+, K^+, Ca^{2+} and Mg^{2+} salts were employed for cations. Similar concentrations of polygalacturonic acid, methanol, iodoacetic acid (IAA) and ethylene diamine tetra acetic acid (EDTA), were used (Fig. 8 and 9). Sodium chloride was found to have activating effect on enzyme activity. At 25 mM concentration; the activity was (142)% of the baseline activity without NaCl. The concentration of 25 mM was highest for maximum pectin methylesterase activity when K^+ was employed. Presence of the bivalent cation (Ca^{2+}) also stimulated enzyme activity. Highest effect of $CaCl_2$ was observed at concentration of 25 mM. Bivalent cations are one of the factors that influence on PME activity by forming non covalent complexes with demethylated pectin, thus, the reaction products were removed. At the concentration of (25) mM $MgCl_2$, the level of inhibition was about 33%. It should be noted that, unlike to pectin methylesterase from higher plants, the effects of cations; on microbial enzyme are not marked.

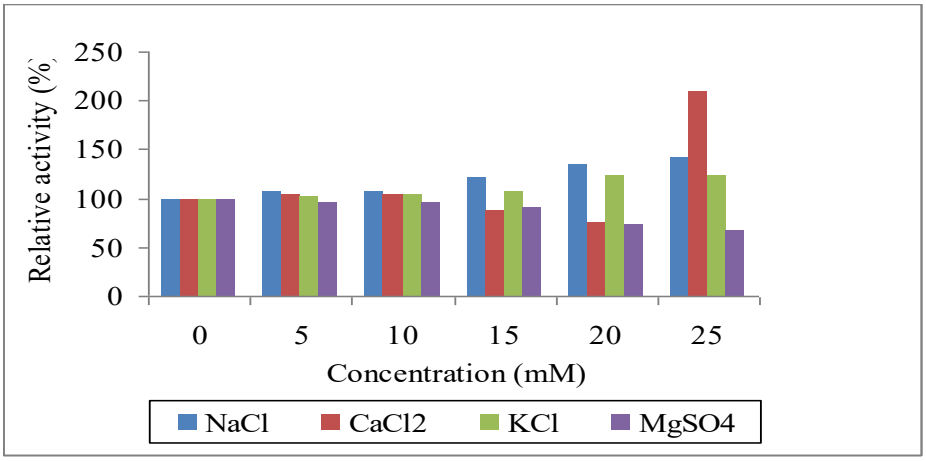

Fig (8): Effect of some salts on activity of PME produced by *A. niger*.

Influence of polygalacturonic acid, methanol, IAA and EDTA on enzyme activity was studied by adding various concentrations of these compounds (5-25 mM), to the reaction mixture prior to assaying enzyme activity. All concentrations of polygalacturonic acid, methanol, IAA and EDTA employed were found to have inhibitory effect to activity of PME (Fig. 9). As seen in the Figure, *A. niger* pectin methylesterase inhibited with 25 mM of each polygalacturonic acid, methanol, IAA and EDTA at the degrees of 37, 51, 60 and 34%, respectively. The inhibitory effect of some chemical compounds used here is in good accordance with results observed with other pectin methylesterases (Vera, *et al*., 2007; Karakus and Pekyardimci, 2009).

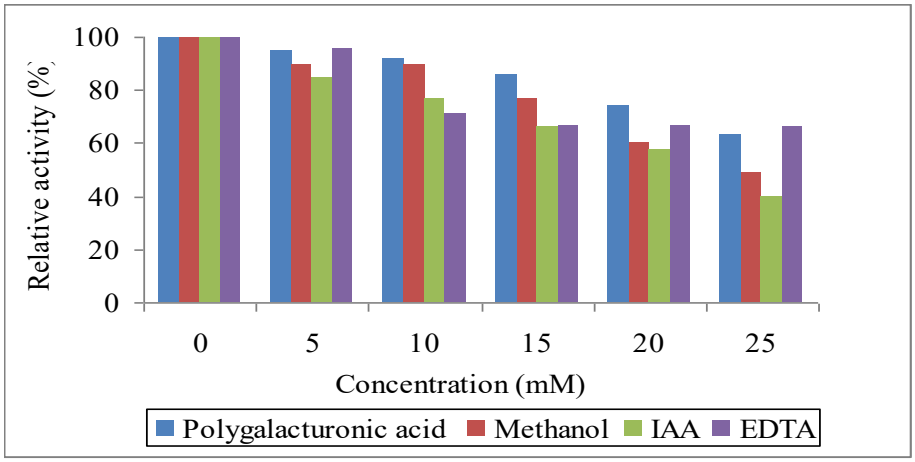

Fig (9): Effect of some chemicals on activity of PME produced by *A. niger*.

Storage stability: Pectin methylesterase solutions were stored at 4 and -18°C for eight weeks. Residual activity was measured every week. The enzyme retained 76 and 87% of initial activity at the end of storage time (8 weeks) at 4 and -20°C, respectively. As could be expected, at -20°C the stability of enzyme was more at 4°C. In general, obtained results were in accordance with other papers as regarding with relatively higher stability of the enzyme at freezing storage and with maintaining a constant activity especially at the first days of storage (Hou *et al*., 1997; Ozler *et al*., 2008).

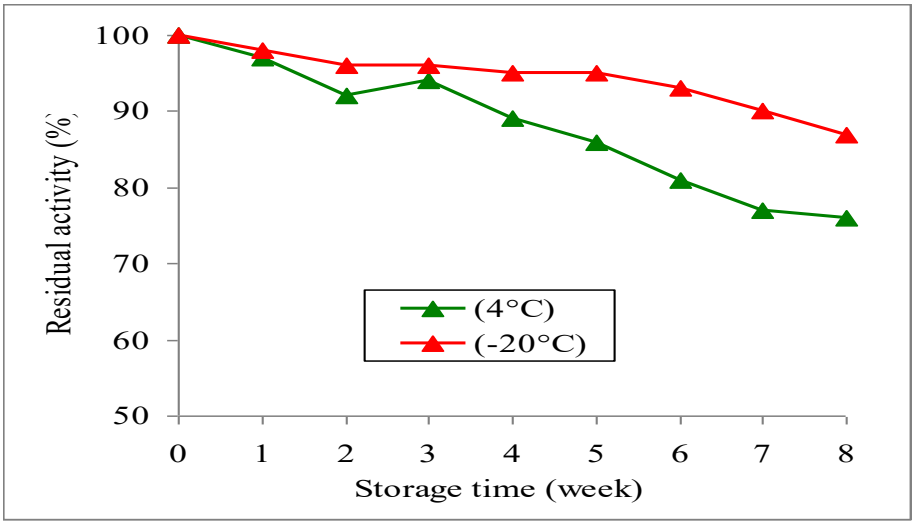

Fig (10): Storage stability of PME produced by *A. niger*.

REFERENCES

Aehle, W. (2004). Enzymes in Industry: Production and Applications. 2nd edit., Wiley-VCH, Inc. New York.

Aizenberg, L., A. Syrchin, A. Sedina, N. Vasilev, N. Shiukareuko, I. Demchenko and N. Vitte (1996). Properties of pectinesterase from *Penicillium fellutanumbiourge* and new developments in pectin applications. Progress Biotechnol., 14: 947-954.

Alebeek, V., V. Scherpenzeel, G. Beldman, H. Schols and A. Voragen (2003). Partially esterified oligogalacturonides are the preferred substrates for pectin methylesterase of *Aspergillus niger*. Biochemical J., 372: 211-218.

Alkorta, I., M. Garbisu, J. Liama and J. Serra (1998). Industrial applications of pectic enzymes: A review. Process Biochem., 33: 21-28.

Arbaisah, S., B. Asbi, A. Junainah, B. Jamilah and J. Kennedy (1997). Soursop pectinesterases: Thermostability and effect on cloud stability of soursop juice. Carbohydrate Polymers, 34: 177-182.

Arotupin, D., F. Akinyosoye and A. Onifade (2008). Purification and characterization of pectin methylesterase from *Aspergillus repens* isolated from cultivated soil. African J. Biotechnol., 7: 1991-1998.

Christgau, S., L. Kofod, T. Halkier, L. Andersen, M. Hockauf, K. Dorreich, H. Dalbo and S. Kauppinen (1996). Pectin methylesterase from *Aspergillus aculeatus*: Expression cloning in yeast and characterization of the recombinant enzyme. Biochem. J., 319: 705-712.

Damodaran, S. (2007). Fennema's Food Chemistry. 4th edit., CRC Press.

Hou, W., Y. Jeong, B. Walker, C. Wei and M. Marshall (1997). Isolation and characterization of pectinesterase from Valencia orange. J. Food Biochem., 21: 309-333.

Huang, A., Y. Wang, H. Yen, C.Jiang and M. Wu (2011). Transacylation properties of pectin methylesterase from *Aspergillus niger*. African J. Food Sci., 5:710-716.

Jaffar, M. and S. Oommen (1993). Production, purification and characterization of pectin methylesterase (PME) from *Arthrobotrys oligospora*. J. Food Biochem., 17: 53-65.

Javeri, H. and L. Wicker (1991). Partial purification and characterization of peach pectinesterase. J. Food Biochem., 15: 241-252.

Jiang, X., P. Chen, M. Yin and Q. Yang (2012). Constitutive expression, purification and characterization of pectin methylesterase from *Aspergillus niger* in pichia pastoris for potential application in the fruit juice industry. 93: 375-381.

Karakus, E. and S. Pekyardimci (2009). Immobilization of apricot pectinesterase (*Prunus armeniaca* L.) on porous glass beads and its characterization. J. Molecular Catalysis B: Enzymatic, 56: 13-19.

King, K. (1991). Characteristics of pectinesterase isolated from bramley apple waste. J. Sci. Food Agric., 57: 43-48.

Maldonado, M., A. Saad and D. Callieri (1994). Purification and characterization of pectinesterase produced by a strain of *Aspergillus niger*. Current Microbiol., 28: 193-196.

Mandhania, S., V. Jain and S. Malhotra (2010). Culture optimization for enhanced production of microbial pectin methylesterase under submerged conditions. Asian J. Biochem., 5: 12-22.

Marangoni, A. (2003). Enzyme Kinetics, a Modern Approach. John Wiley and Sons. New York.

Mutlu, M., K. Sarioglu, N. Demir, M. Ercan and J. Acar (1999). The use of commercial pectinase in fruit juice industry. part 1: Viscosimetric determination of enzyme activity. J. Food Eng., 41: 147-150.

Nchienzia, H., R. Morawicki and V. Gadang (2010). Processing, products, and food safety enzymatic hydrolysis of poultry meal with endo and exopeptidases. Poultry Sci., 89: 2273-2280.

Ozler, A., E. Karakus and S. Pekyardimci (2008). Purification and biochemical characteristics of pectinesterase from Malatya apricot (*Prunus armeniaca* L.). Preparative Biochem. Biotech., 38: 358-375.

Sartoglu, K., N. Demir, J. Acar and M. Mutlu (2001). The use of commercial pectinase in the fruit juice industry. part 2: Determination of the kinetic behavior of immobilized commercial pectinase. J. Food Eng., 47: 271-274.

Simpson, B. (2012). Food Biochemistry and Food Processing. 2ndedi., Wiley Blackwell. New York.

Vera, M., J. Montoya, G. Calva and E. Ramirez (2007). Extraction, thermal stability and kinetic behavior of pectin methylesterase from hawthorn (*Crataegus pubescens*) fruit. LWT., 40: 278-284.

توصيف إنزيم البكتين مثيل استريز من *Aspergillus niger*

عدي حسن الجماس وصالح حمادي سلطان ووليد احمد محمود

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المستخلص

كان الغرض من هذه الدراسة هو تقييم الصفات المختلفة لإنزيم البكتين مثيل استريز المنتج من فطر *Aspergillus niger*. بلغ الوزن الجزيئي للأنزيم 59.668 كيلو دالتون. كانت درجة الحرارة المثلى لفعالية الإنزيم 45°م. فقد الإنزيم معظم فعاليته لدى تحضينه بدرجة حرارة 75°م لمدة 20 دقيقة. وصلت أقصى فعالية للإنزيم عند الأس الهيدروجيني 4 وأظهر الإنزيم ثباتا عاليا عند الأس الهيدروجيني 4 مع إحتفاظه بأكثر من 90٪ من فعاليته بعد عشر دقائق من تحضينه على أس هيدروجيني يعادل 5، بلغت قيمة ثابت ميكيلس (Km) 1 ملغم/مل. بلغت قيمة طاقة التنشيط للتفاعل الحيوي المحفز بفعل الإنزيم محسوبة على أساس 9.66 كيلوسعرة/مول. فقد الإنزيم 24 و 13٪ من فعاليته الأصلية بعد شهرين من الخزن على درجتي الحرارة 4 و 20-°م، وعلى التوالي. كان لوجود كلوريد الصوديوم والبوتاسيوم والكالسيوم بتركيز (25) مليمولاري في وسط التفاعل تأثير تنشيطي للإنزيم، فيما أدى وجود كبريتات المغنيسيوم وحامض الكالاكتيورونيك المتعدد والميثانول و IAA و EDTA بنفس التركيز إلى تثبيط الإنزيم وبدرجات متفاوتة.

الكلمات المفتاحية: توصيف، انزيم البكتين، مثيل استريز، *Aspergillus niger*