

## DETERMINATION OF PECTIN METHYLESTERASE ACTIVITY OF PRICKLY PEAR (*OPUNTIA FICUS INDICA*) FRUIT AND ITS KINETIC PARAMETERS

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### ABSTRACT

The prickly pears (*Opuntia ficus indica*) belonging to the Cactaceae family are grown in arid and semi-arid regions and harvested in summer to fall season in the Mediterranean basin, the United States, and South America. Besides having pleasure flavor, high betalain pigment, vitamin C, mineral content, and amino acids such as proline and taurine are made prickly pear as very useful functional food or food ingredient. In this study, biochemical properties (optimum temperature, optimum pH, the maximum reaction rate ( $V_{max}$ ), substrate specificity ( $K_m$ ), and thermal stability) of the Pectin methylesterase (PME) obtained by partial purification from prickly pear were investigated. PME activity was measured by titrimetric method and apple pectin was used as substrate for calculations. The enzyme has optimum activity at pH 7.0 and an optimum temperature of 40 °C.  $K_m$  and  $V_{max}$  values of the enzyme were calculated as 0.162 mg/mL and 3.05 units/mL, respectively. Activation energy ( $E_a$ ) and value of  $Z$  are calculated as 57.86 kJ.mol<sup>-1</sup> and 41.32 °C, respectively. In thermal inactivation studies at 70, 80, and 90 °C reaction rate constants ( $k$ ) were found as 0.16, 0.23, and 0.50 min<sup>-1</sup>, thermal half-life times ( $t_{1/2}$ ) were calculated as 4.35, 3.08, and 1.42 min, and decimal reduction time ( $D$ -value) was calculated as 14.44, 10.25 and 4.74 min, respectively. In addition, by analyzing the biochemical properties of the PME enzyme found in prickly pear fruit, researchers expect to gain insights into the ideal processing temperature and other factors that impact the quality of the fruit when it is processed into different food products.

**Keywords:** *Opuntia ficus indica*, prickly pear, pectin methylesterase, kinetics parameters, thermal inactivation

### INTRODUCTION

A member of the cactus genus *Opuntia* and a member of the Cactaceae family, the prickly pear (*Opuntia ficus indica*) is a perennial succulent native to arid and semi-arid regions where water for cultivation is limited. The prickly pear is the most productive species of the genus *Opuntia* and its fruits have a very delicious aroma. The homeland of the prickly pear, which is known to be of Mexican origin, is the American continent. In addition, Mexico ranks first in the world for the production and consumption of prickly pears. It is known that Christopher Columbus, with the discovery of the New World, brought it to Spain and from there spread to other Mediterranean countries (Fernández-López *et al.*, 2002; Messina *et al.*, 2021). Sicily and Calabria, for instance, are home to the majority of the *ficus indica* plant in Italy (Maniaci *et al.*, 2024). More than thirty countries, including those in Africa, Asia, and southern Europe, cultivate this cactus for food and medicinal purposes (Demir *et al.*, 2023). In addition to its medicinal uses due to its positive health effects, prickly fig is also used as a colourant for food, juices, liquors, confectionery, and animal nutrition (Livera-Muñoz *et al.*, 2024). The prickly pear, which has orange-yellow colors, is generally harvested between August and September in Türkiye. Although the composition of the fruit varies according to the plant variety and environmental conditions, in general, the fruit contains approximately 45-67% pulp, 33-55% prickly skin, and 2-10% seeds. Edible fruit pulp has a water content of 84-90%, a water-soluble dry matter content of 12-17%, and a pH value of 5.3-7.1. The titratable acidity rate in terms of citric acid is in the range of 0.05-0.18%, and it has been reported to be among the foods with low acidity (Piga, 2004; Saenz, 2000). Carotenes, phenolic compounds, and antioxidants are all key nutrients found in the *Opuntia ficus indica* plant. It also contains large amounts of betanin, indoxanthin, vitamin C, magnesium, calcium, phosphorus, fiber (lignin, cellulose, hemicellulose) and free amino acids (proline, glutamine, taurine). Since these bioactive compounds give the fruit functional properties such as antioxidant, hypoglycemic, hypolipidemic, hypocholesterolemic, anti-cancerogenic, and anti-inflammatory, it has been utilized for a variety of medicinal purposes in folk medicine. It has been reported to help in the treatment of a variety of chronic illnesses. It is, furthermore, used in pharmaceuticals or cosmetic industries (Barba *et al.*, 2020; Çakmak *et al.*, 2020; Medina *et al.*, 2007; Silva *et al.*, 2021). In addition, the main issue in the production and marketing of prickly pears is the short harvest season and high perishability, which leads to post-harvest losses of up to 60% (Cruz-Cansino *et al.*, 2016). The species, growth stage, maturity, harvest period, and postharvest

handling all affect the chemical and nutritional makeup of prickly pears. Prickly pears' nutritional, practical, and medicinal qualities have made them one of the most significant crops in recent years from an economic standpoint. They exhibit significant promise for use in pharmaceutical, cosmetic, and environmental applications (Aparicio-Ortuño *et al.*, 2024).

Pectin plays an important role in the hard and firm structure of plant tissues such as fruits and vegetables. In particular, it plays a role in the ripening of fruits and in the growth of the cell wall during cell development. Its role in ripening is to form low methylation pectin. Its role in cell wall growth is to induce autolysis and local pH reduction, which activates the enzymes involved in growth. Therefore, changes in the structure of pectin have very important consequences in terms of texture. For example, the enzymatic breakdown of pectin and other cell wall polysaccharides during fruit ripening causes the fruit to soften. In particular, tomato paste made from tomatoes with degraded pectin does not have a sufficient consistency. This breakdown of the pectin molecule is caused by pectin enzymes, of which there are many subgroups. Pectic enzymes can be divided into pectinesterases (ester bond hydrolyzing enzymes) and depolymerases (chain-breaking enzymes) according to their effect on the galacturonan backbone of the pectin molecule. These different breaking reactions are used to classify pectinases (Ackerley & Wicker, 2003; Anthon & Barrett, 2012; Pelloux *et al.*, 2007; Sila *et al.*, 2009).

The control of pectin methylesterase (PME) activity is very important for biotechnological processes related to the production and preservation of fruit juices and purees. For instance, it is difficult to achieve turbidity stability in citrus juices or fruit nectars due to pectin modification, and these products can experience problems with serum separation within a short period of time. Furthermore, these enzymes play a very important role in the changes that occur in fruits and vegetables during post-harvest storage. Therefore, there is a need to determine the activity of pectin-degrading enzymes in the raw material or in the end product (Pelloux *et al.*, 2007; Sila *et al.*, 2009).

With the understanding of the relevance of the PME enzyme, many researchers have conducted studies to determine the properties of the PME enzyme in a variety of fruits and vegetables. For this reason, PME has been examined in numerous foods, such as tomatoes (Anthon and Barrett, 2012), hawthorn (Vivar-Vera *et al.* 2007), apricot (Ünal and Şener, 2013), carrot (Sila *et al.* 2007), strawberry (Ly-Nguyen *et al.* 2002), guava (Leite *et al.*, 2006), persimmon (Alonso *et al.* 1997), and grapefruit (Guiavarc'h *et al.* 2005). However, there is no study in the current literature review to purify and characterize the PME enzyme, which has the potential to be found in prickly pear fruit.

The objective of this research was to identify and isolate various biochemical properties, kinetics, and thermal stability of the PME enzyme found in prickly pear fruit. It is anticipated that this study will contribute new knowledge to the existing literature on prickly pear. Furthermore, understanding the biochemical properties of the PME enzyme extracted from prickly pear fruit is crucial for the advancement of biotechnological processes related to the preservation and storage of fruit and vegetable juices. In addition, by analyzing the biochemical properties of the PME enzyme found in prickly pear fruit, researchers expect to gain insights into the ideal processing temperature and other factors that impact the quality of the fruit when it is processed into different food products.

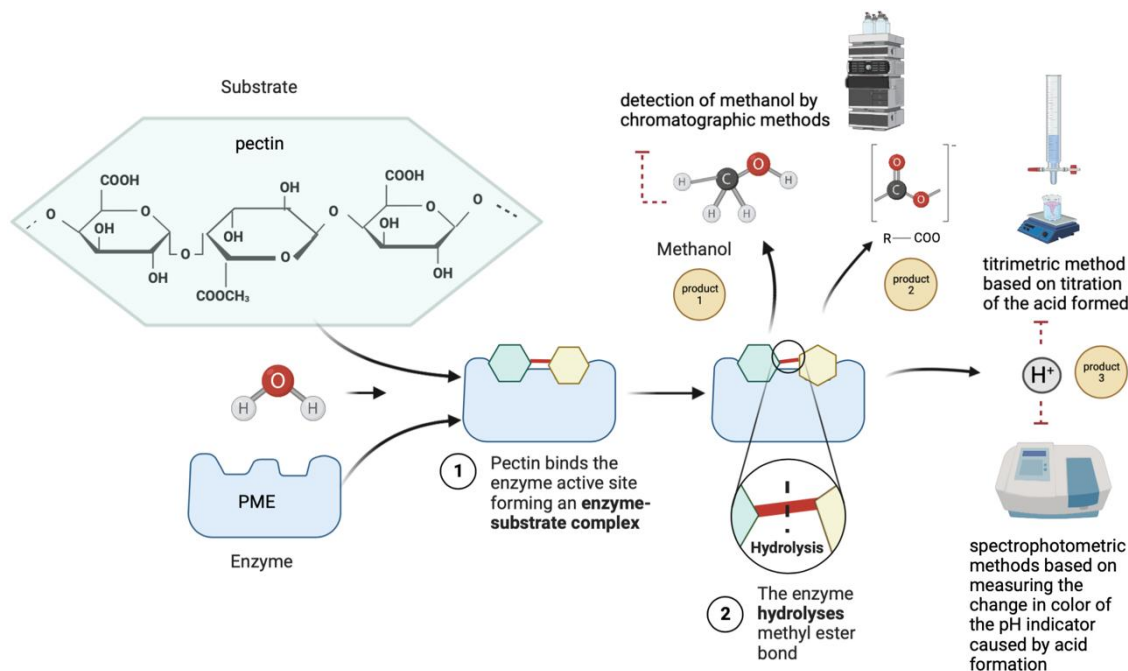
**MATERIAL AND METHODS**

The prickly pear samples used in this study were collected from Silifke district in the province of Mersin in Türkiye and frozen at -25 degrees Celsius for analysis.

Apple pectin was obtained from Sigma and all the chemicals used were of analytical grade.

**Extraction of the PME enzyme and determination of its activity**

The methods for measuring PME activity are based on measuring the products formed as a result of the reaction as shown in Figure 1. For example, the activity of PME may be determined by the chromatography of methanol, one of the products obtained by the reaction (Zainol and Ismail, 2019). The most common methods to determine the activity of PME are titrimetric methods based on titration of the acid produced and spectrophotometric methods based on measuring the change in the colour of the pH indicator, such as bromimetonol blue, caused by the decrease in the pH of the medium due to acid formation (Ackerley & Wicker, 2003; Hagerman & Austin, 1986; Zimmerman, 1978).



**Figure 1** Methods for measurement of pectin methylesterase (PME) activity

The prickly pear samples, peeled and frozen, were measured at +4 °C with a ratio of 1:4 (w/v) of solvent (1 M NaCl) and homogenised for 1 min in a blender, then passed through a filter paper. The homogenate, called crude extract, was centrifuged at 4 degrees Celsius and 10,000 x g for 30 min to remove the solid fractions. Immediately after extraction, the activity of PME was measured by titrimetric assay. In the titration, the pH was first adjusted to 7.0 by using 0.1 N sodium hydroxide as substrate, followed by approximately 20 ml of 0.5 percent (w/v) pectin solution prepared with 1 M sodium chloride. After adding 0.5 ml of extract to the solution, 0.005 N NaOH was recorded at 10 and 20 min at a steady state pH of 7.0. Then 0.005 N NaOH spent was used to calculate (Saenz, 2000; Ünal and Bellur, 2009). As shown in Figure 2, in order to titrate at 30 °C, a pump was placed in the water bath at 30 °C in order to circulate the water. Then titration was performed in a double-walled glass chamber at constant temperature and stirred with a magnetic stirrer.



**Figure 2** Assay of enzyme activity by using the titrimetric method

Then, the alkaline consumption was recorded over time and the enzyme activity calculated from equation 1, as shown below (Zimmerman, 1978).

$$PME \left( \frac{Units}{g} \right) = \frac{Spent\ NaOH\ (ml) \times Normality\ (NaOH) \times 1000}{Time\ (min) \times extract\ (g)} \quad (1)$$

**Optimum pH**

The pH of the 0.5 percentage point (w/v) apple pectin solution prepared with 1M sodium chloride was adjusted by adding 0.1 N NaOH solution to the range 4-9. Consumption of 0.005 N NaOH was observed after adding 0.5 mL of enzyme to maintain the pH adjustment at 10 and 20 min. The highest observed activity was accepted as 100 percent and activity at other pH values was compared to the highest activity and the relative activity of the enzymes was calculated. The pH of the PME enzyme that shows the highest activity has been defined as the optimum pH (Ünal and Bellur, 2009; Zimmerman, 1978). After the optimal pH value has been determined, further testing at this pH value was performed.

**Optimum temperature**

In order to determine the temperature at which prickly pear PME showed the highest activity, the activity of the enzyme was measured in the temperature range from 20 to 70 °C. A 0.5% (w/v) apple pectin solution made with 1M NaCl at the previously determined optimal pH was used (Ünal and Bellur, 2009; Zimmerman, 1978).

**Determination of kinetic parameters**

The Michaelis-Menten constant (Km) of the substrate affinity and the maximum velocity (Vmax) of the PME were calculated by the use of different concentrations of pectin solutions at the pH and temperature optimal values. The solution of pectin used as substrate was prepared at a concentration of between 0.1 and 4 g per L. The enzyme Km and Vmax values were calculated by plotting 1/V vs. 1/S using the Lineweaver-Burk method (Ünal and Bellur, 2009; Zimmerman, 1978).

**Thermal inactivation**

To determine the effect of temperature on the enzyme, the enzyme solution was exposed to varying temperatures (70, 80 and 90 degrees Celsius) and time periods (1, 3, 5, 7, 10, 15, 20 and 30 minutes). Two replicates were produced in two parallel tubes in a sealed glass chamber in a water bath at the specified temperatures and

times. The tubes were pre-heated to a specified temperature before the enzyme was added. At the end of this time the sample of enzyme was transferred into the tube and, for a specified period, subjected to the appropriate temperature. After the desired time, the tube was quickly removed from the water bath and refrigerated in a refrigerator. When samples have reached room temperature, residual activity has been measured. For the determination of values of  $k_D$  (inactivation constant,  $\text{min}^{-1}$ ),  $t_{1/2}$  (half-life, min),  $E_a$  (activation energy,  $\text{kJ/mol}$ ),  $Z$  (temperature change which may cause a 10-fold change in the reaction rate constant ( $k$ ),  $^{\circ}\text{C}$ ), and  $D$  (decimal reduction time, min) values, the residual activity ( $A_t$ ) of the enzyme exposed was compared with that of the enzyme not exposed to temperature ( $A_0$ ). (Ünal and Bellur, 2009; Zimmerman, 1978).

**Statistical analysis**

All experiments were performed in triplicates and data were presented as mean  $\pm$  standard deviation. The statistical analysis was conducted by means of a one-way ANOVA using SPSS Version 26. A p value of less than 0.05 was considered as indicating a statistically significant difference between samples (\*  $P < 0.05$ , \*\*  $p < 0.01$ ).

**RESULTS AND DISCUSSION**

**Optimum pH**

Optimum pH is the pH or pH range in which the enzymes show the highest reaction rate. Below and above this optimum pH, the enzyme activity can decrease. Optimum pH values of enzymes can vary depending on the type and ripeness of the fruit, as well as the substrates it contains and the extraction method used. In order to determine the galacturonic acid formation as a blank titration, titration was recorded at ambient conditions without adding enzyme extract at each pH value. Spontaneous demethylation (galacturonic acid formation) has been observed in basic media at pH 8.0 and 9.0. Actual consumption was used to calculate PME activity by calculating the difference between blind consumption due to spontaneous demethylation and total consumption (Gurrieri et al. 2000). The pH with the highest activity is taken as 100 percent and the results are shown in Graph (figure) 3 in terms of relative activity. The 10th and 20th minute measurements showed that the optimal pH value of PME obtained from prickly pear fruit was 7.0, similar to the PME obtained from grapefruit (*Citrus paradisis*) using pectin as substrate. (Guiavarc'h et al., 2005). As can be seen in figure 3, an acute drop in enzyme activity was observed above pH 7.0. Regarding the optimal pH of PME for different fruits, it was found to be 7.5, 7.4, 8.0 and 8.5 for plum (*Prunus domestica*) (Nunes et al. 2006) persimmon (*Diospyros kaki*) (Alonso et al. 1997) carrot (*Daucus carota* var. Nerac) (Sila et al. 2007) and guava (*Psidium guajava* L.) (Leite et al., 2006).

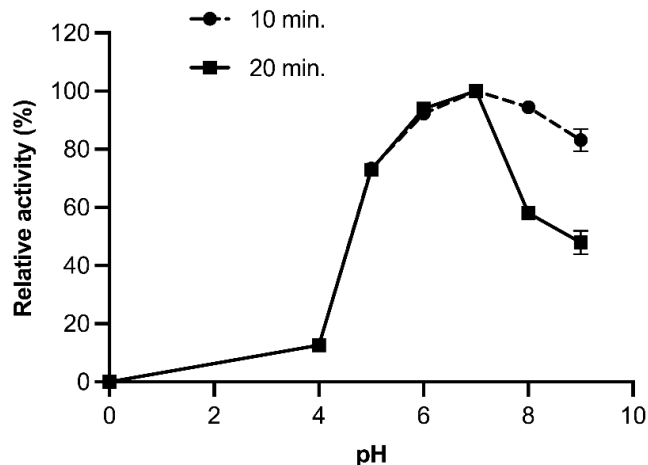


Figure 3 Effect of pH on prickly pear PME activity

**Optimum temperature**

High temperatures may inactivate the enzyme. The temperature at which the rate of reaction reaches its maximum value is called optimal temperature. The optimum activity of most enzymes is at 30-40 degrees Celsius; denaturation may start at a temperature higher than 45 degrees Celsius. (Ünal and Bellur, 2009). In our study, activity measurements were carried out at temperatures between 20 and 70 degrees Celsius to determine the optimal temperature value for PME from prickly pear fruit. The optimal temperature value of prickly pear PME was observed to be 40  $^{\circ}\text{C}$  as shown in figure 4. As observed, the activity increased proportionally with the increase in temperature from 20  $^{\circ}\text{C}$  to 40  $^{\circ}\text{C}$  and decreased again at higher temperatures. Optimum temperature values of PME obtained from different plants were found to be close to the optimum temperature values of prickly pear PME. The optimum temperature value of prickly pear PME is lower than strawberry

*Fragaria ananassa*, cv Elsanta (60  $^{\circ}\text{C}$ ) (Ly-Nguyen et al. 2002) and apple *Golden Delicious* (63  $^{\circ}\text{C}$ ) (Denè et al. 2000) fruits, while it is higher than papaya *Carica papaya* (35  $^{\circ}\text{C}$ ) (Lim and Chung, 1993) fruit.

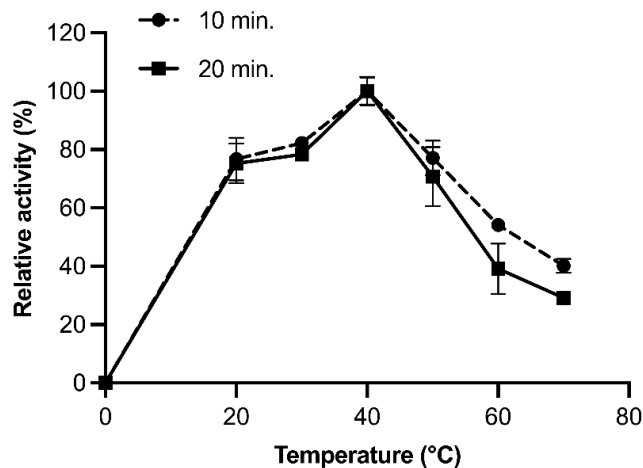


Figure 4 Effect of temperature on prickly pear PME activity

**Kinetic parameters**

Pectic substances are the most important natural substrates for PME found in fruits and vegetables. Kinetic measurements were made using Lineweaver-Burk graphic method at optimum pH (7.0) and optimum temperature (40  $^{\circ}\text{C}$ ) determined by using apple pectin. The Lineweaver-Burk plot of the prickly pear PME is shown in figure 5. The specific substrate concentration ( $K_m$ ) was calculated as 0.162  $\text{mg/mL}$  ( $r^2=0.963$ ) as a measure of the enzyme's affinity for the substrate in prickly pear. It can be said that the smaller the  $K_m$  value, the higher the affinity of the enzyme to the substrate (Van Boekel, 2008). The  $V_{max}$  value representing the maximum reaction rate of PME from prickly pear was also calculated to be 3.05 units/mL, similar to the PME activity obtained from black carrot (3.75 units/mL) (Ünal and Bellur, 2009) by the titrimetric method. Since both  $K_m$  and  $V_{max}$  values vary with source, temperature, salt concentration, and pH of the reaction medium, there is a wide range of values in the literature (Van Boekel, 2008). In the literature review, the affinity of PME obtained from prickly pear to the substrate is lower than PME obtained from carrot (0.04  $\text{mg/mL}$ ) (*Daucus carota*) (Balogh et al. 2004) and apple (0.098  $\text{mg/mL}$ ) (*Golden* sp.) (Denè et al. 2000), while it is higher than PME obtained from hawthorn (2.82  $\text{mg/mL}$ ) (*Crataegus pubescens*) (Vivar-Vera et al. 2007) fruit, and pepper (0.329  $\text{mg/mL}$ ) (*Capsicum annum*) (Castro et al., 2006).

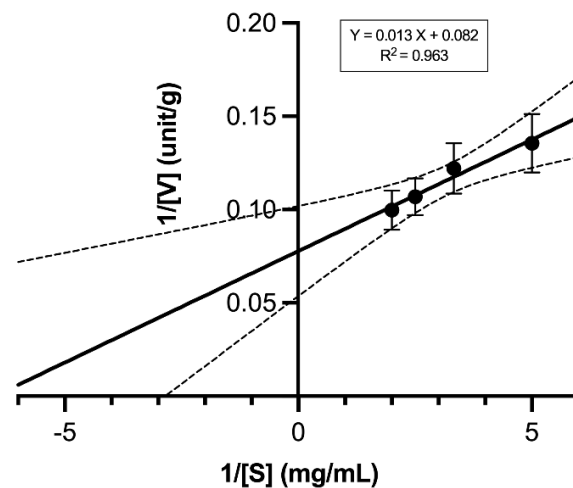
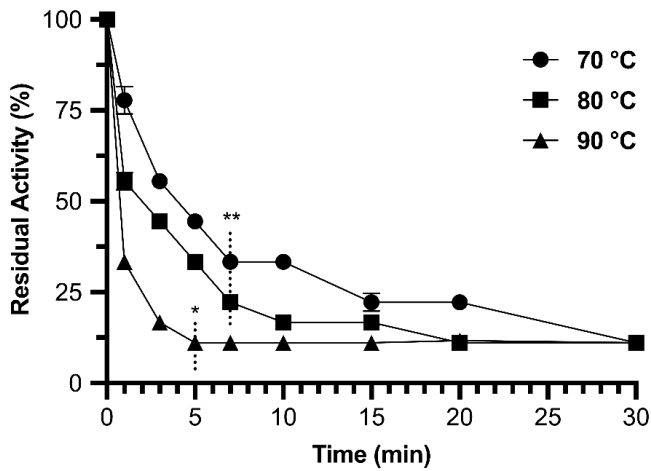


Figure 5 Lineaweaver-Burk diagram for PME activity with the pectin substrate

**Thermal inactivation**

Thermal inactivation treatments of PME were performed in a temperature range of 70 to 90  $^{\circ}\text{C}$  using a water bath. In this temperature range, PME inactivation showed different behaviors in inactivating the enzyme over time. As can be seen in figure 6, enzyme inactivation was faster at 90  $^{\circ}\text{C}$  than at 70 and 80  $^{\circ}\text{C}$ . At 90  $^{\circ}\text{C}$  the residual activity remained at the lowest level after 5 minutes, while at 70 and 80  $^{\circ}\text{C}$  there was no significant change after 7 minutes. This suggests that at high temperature the enzymes are rapidly denatured, as Vivar-Vera emphasized in his

study. (Vivar-Vera et al., 2007). Since the thermal stability profile was as shown in figure 6, these times were used in figure 7a to determine the reaction rate constant at different temperatures. Such an inactivation profile of PME has also been reported for acerola and mango extracts (De Assis et al. 2000); (Diaz-Cruz et al. 2016) and pectin esterase (PE) for apricot (Özler et al. 2008).



**Figure 6** Thermal stability profile of prickly pear PME crude extracts at different temperatures, (\*...): line showing which times should be used in the determination of the reaction rate constants (k)

Thermal inactivation parameters were calculated by comparing the activities of the enzymes exposed to heat treatment with the activity of the unheated sample. The rate of reaction of thermal inactivation of enzymes, expressed in terms of first-order reactions, is expressed in the following equation 2. If the plot of  $\ln(A/A_0)$  versus time is plotted at a constant temperature, this is obtained in figure 7a diagram shown below. The half-life ( $t_{1/2}$ ) was calculated by the equation given in equation 3 (Van Boekel, 2008).

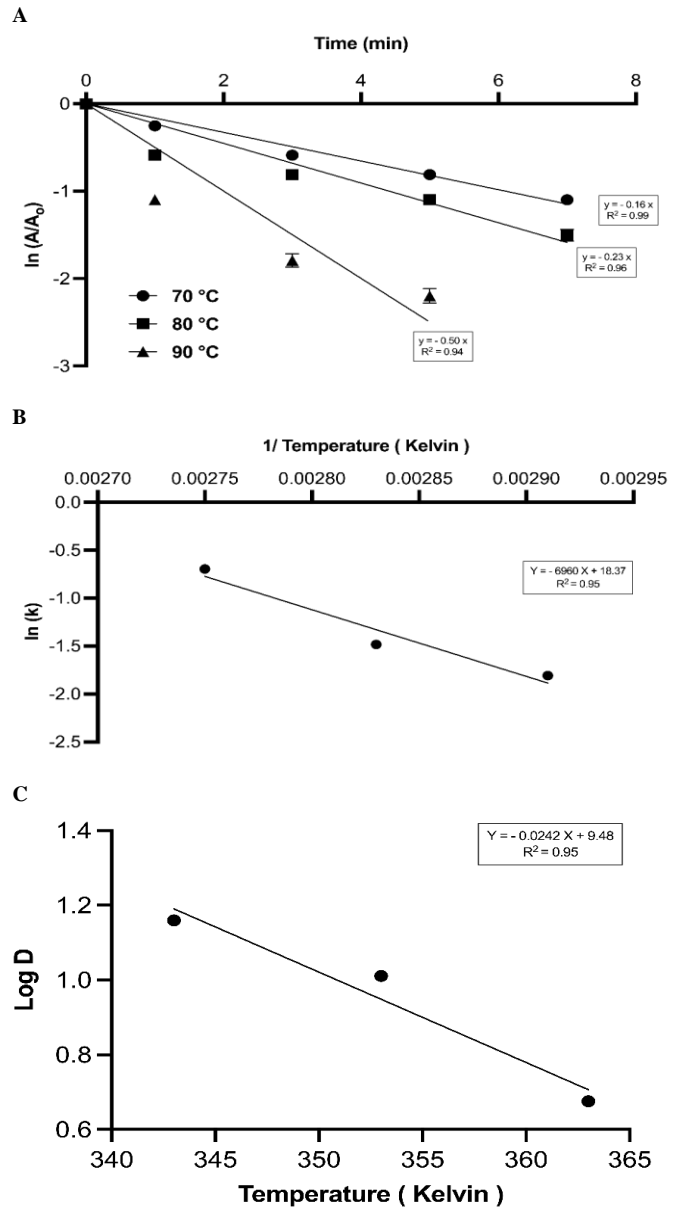
$$A_t = A_0 e^{-kt} \quad (2)$$

$$t_{1/2} = \frac{0.693}{k} \quad (3)$$

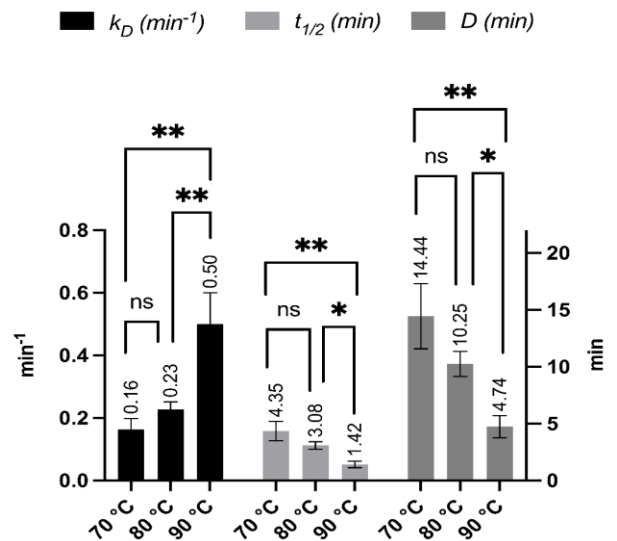
$$D = \frac{2.303}{k} \quad (4)$$

The activation energy ( $E_a$ ), required energy for heat inactivation of PME from prickly pear, was calculated to be 57.86 kJ/mol by the Arrhenius equation and graph (Fig. 7b). Some of the reported  $E_a$  values include 196.8 kJ/mol for black carrot PME (Ünal and Bellur, 2009), 245.6 kJ/molK for mango PME (Diaz-Cruz et al. 2016), 78.2 kJ/mol for pineapple juice PME (Cautela et al. 2018), 62.61 kJ/mol for sour orange juice PME (Aghajanzadeh et al., 2016). If the plot of the log D values is plotted against the temperature values (70, 80 and 90 °C), a plot like that in figure 7c is obtained and the slope of the line gives the value 1/Z. The Z value, defined as the temperature range between which the D-value changes tenfold, was calculated to be 41.3 °C for prickly pear PME. This value is close to the Z value of PME obtained from orange juice with 36.9 °C, but higher than that derived from mango, acerola, apple and apricot (Aghajanzadeh et al., 2016; De Assis et al., 2000; Denè et al., 2000; Diaz-Cruz et al., 2016; Ünal and Şener, 2013).

The reaction rate constants ( $k$ ), the half-life ( $t_{1/2}$ ), and the decimal reduction time ( $D$ -values) of prickly pear PME for 70, 80, and 90 °C were given in figure 8. The higher the inactivation rate constant, the less thermostable the enzyme. Therefore, as can be seen in figure 6, the enzyme is less stable at higher temperatures (Özler et al., 2008). The thermal inactivation rate constants of prickly pear PME were found to be 0.16, 0.23, and 0.50  $\text{min}^{-1}$  for 70, 80, and 90 °C, respectively, and the half-life times to be 4.35, 3.08, and 1.42 min, respectively. The  $D$  value, time to inactivate 90% of the enzyme, calculated using equation 4 was found to be 14.44 min at 70 °C, 10.25 min at 80 °C, and 4.74 min at 90 °C. As can be seen from figure 8, the reaction rate constants ( $k$ ) values increased as the temperature increased. Nevertheless, the half-life ( $t_{1/2}$ ) and  $D$  values decrease with increasing temperature. When the inactivation parameters of PME are compared with each other, there is no statistically significant difference ( $p > 0.05$ ) in the reaction rate constant ( $k$ ), half-life ( $t_{1/2}$ ) and decimal reduction time ( $D$  values) obtained at 70 and 80 °C. The difference between ( $t_{1/2}$  and  $D$  values at 70 and 90 °C is more significant ( $p < 0.01$ ) than at 80 and 90 °C ( $p < 0.05$ ). If we look at the ( $k$ ) values, we see that there is a clear difference between those obtained at 70-90 °C and 80-90 °C. The high heat treatment at 90 °C was effective in this difference.



**Figure 7** Determination of the temperature influence on the enzyme. (A) Logarithmic plot of the residual activity of the crude of prickly pear PME extract. (B) Arrhenius plots for a crude prickly pear PME extract. (C) Plots of log D versus absolute temperature for a crude prickly pear PME extract



**Figure 8** Inactivation parameters of prickly pear PME. Values are expressed as the mean  $\pm$  SD. A p value below 0.05 was considered to indicate a statistically significant difference. Nonsignificant (ns) denotes  $P > 0.05$ , \*denotes  $p < 0.05$ , and \*\*denotes  $p < 0.01$

In the PME enzyme study of pineapple juice by Cautela et al. (2018), and sour orange juice by Aghajanzadeh et al. (2016), they found the reaction rate constants ( $k$ ) values as 0.54, 1.26, and 2.28  $\text{min}^{-1}$  for pineapple, 0.03, 0.05, and 0.1  $\text{min}^{-1}$  for orange at 70, 80, and 90 °C, respectively. They also reported that  $D$  values of PME from pineapple juice were 4.1, 1.81, and 1 min, and for sour orange juice 77.3, 49.5, and 22.1 min at 70, 80, and 90 °C, respectively. Compared to our values at the same temperatures, the  $D$  values of PME from prickly pear were lower than orange juice and higher than pineapple juice. In a study carried out by Ünal and Bellur, (2009), half-life ( $t_{1/2}$ ) values at 55, 60 and 65 °C for PME from black carrots were given as 17.4, 5.2 and 2.1 min, respectively. Ünal and Şener, (2013) studying the thermal inactivation of apricot PME noted ( $k$ ) values of 0.04, 0.13 and 0.32  $\text{min}^{-1}$  at 60, 65 and 70 °C, respectively, which correspond to the in are similar to those obtained in this study. These results show that the thermal stability and kinetic parameters of PME depend on the treatment method used, the fruit variety and the different isoforms of the enzyme (Aghajanzadeh et al., 2016). The results presented in this study were within the range of the literature values.

## CONCLUSION

In this study, the kinetic and thermal stability properties of the crude extract of pectin methylesterase enzyme (PME) obtained from prickly pears were investigated. At the end of the research, optimum pH value, optimum temperature, specific substrate concentration, and thermal inactivation kinetic parameters of prickly pear PME enzyme were determined. The optimum pH and temperature values for PME activity were found to be 7.0 and 40 °C, respectively. The  $K_m$  and  $V_{max}$  values of the PME enzyme were found to be 0.162 mg/mL and 3.05 units/mL, respectively. According to the thermal inactivation results, the reaction rate constants ( $k$ ) at 70, 80 and 90 °C were noticed 0.16, 0.23, and 0.50  $\text{min}^{-1}$ , respectively. Half-life ( $t_{1/2}$ , min) at 70, 80, and 90 °C were 4.35, 3.08, and 1.42 min, respectively, while  $D$  values were 14.44, 10.25 and 4.74 min. The activation energy ( $E_a$ ) and  $Z$  values for prickly pear PME were found to be 57.86 kJ/mol and 41.32 °C, respectively.

Different studies indicate that it is acceptable to process prickly pear into a new product such as fruit juice. Despite the short harvest time of the prickly pear fruit and the problems in preserving the fruit, it is believed that processing into various products will be beneficial to meet increasing consumer demand. Therefore, control of PME activity is of paramount importance in all biotechnological processes focused on monitoring and storage of fruit and vegetable juices. In addition, it is anticipated that determining the biochemical properties of the PME enzyme isolated from prickly pear fruit, which has the potential to process various foods, will provide an indication of processing temperature and other parameters affecting fruit quality can processing the fruit into various foods.

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