Thermal Inactivation Kinetics of Pectin Methyl Esterase (PME) in Apple, Mango and Cauliflower

El-Shamei, Z. A. S., Amal A. Gab-Alla, A. A. Shatta and A. M. M. Rayan
Food Technology Department, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

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Abstract: Thermal inactivation kinetics of pectin methyl esterase (PME) in apple, mango and cauliflower extracts have been determined at temperatures ranged from 50 to 90°C. The kinetics of PME from the three extracts showed a first-order model. At 70°C, D values were 1.7, 23.8 and 2.3 min., z values were 7.91, 17.67 and 10.64°C and activation energies (Ea) values were 272.24, 126.7 and 208.42 kJ mol^{-1} for apple, mango and cauliflower, respectively. These data indicated that mango PME was the more heat-stable, while apple PME was the lowest one.

Keywords: Thermal inactivation, pectin methyl esterase, apple, mango, cauliflower.

INTRODUCTION

Pectin methyl esterase (PME; EC: 3.1.1.11) is one of the hydrolyzing enzymes realizing prime important to the food industry. Also known as pectin esterase, catalyses hydrolysis of the methoxyl group of pectin, forming pectic acid as a product of the reaction. This enzyme acts preferentially on the methyl ester group of the galacturonate unit next to the non-esterified galacturonate unit. The decrease in the degree of pectin methoxylation, may in turn, trigger different processes related to texture and firmness (Tijskens *et al.*, 1999). Such a catalytic action makes PME one of the most important enzyme in the food industry for protecting and improving the texture and firmness of several fresh or processed fruits, vegetables and juices or other industrial products that involve the presence or absence of intact pectin (Alonso *et al.*, 1997). In all cases, the inactivation of PME is required for cloud stability of cloudy plant juices. This is usually achieved by thermal treatments, such as pasteurization or High Temperature Short Time (HTST) treatment. It is widely accepted that these treatments are sufficient to inactivate PME.

Denes *et al.* (2000) found that isoelectric points of the extracted and purified PME from apple (cv Golden Delicious) were higher than 9. Kinetic parameters of the enzyme were determined as K_m= 0.098 mg/ml and V_{max}=3.86 μ mol/min/ml of enzyme. The optimum pH of the enzyme was above 7.5 and its optimum temperature was 63°C. The purified PME required the presence of NaCl for optimum activity, and the sodium chloride optimum concentration increased with decreasing pH (from 0.13 M at pH 7 to 0.75 M at pH 4). The heat stability of purified PME was investigated without and with glycerol (50%), and thermal resistance parameters (D and z values) showed that glycerol improved the heat resistance of apple PME.

Yi *et al.* (2004) applied the first-order kinetic model of a 2-component system to investigate the thermal pasteurization of mango juice. Thermostable and thermolabile pectinesterase (PE) components (41 and 59%, respectively) were detected in the mango juice. The average z values of thermostable (Zs) and thermolabile (Zl) were 14.65 and 15.94°C, respectively, within the temperature range of 80-85°C. The calculated and experimental linear thermal pasteurization curves for mango juice were in good agreement at a wide range of temperature (80-90°C).

PME from Brazilian guava showed two isoenzymes (conc PME 70% saturation by ammonium sulphate) and Iso4 PME, one of the isoforms from gel filtration with the greatest specific activity) differing in their molecular mass values. The optimum pH of the enzyme (for both samples) was 8.5 and the optimum temperature ranged from 75 and 85°C. The optimum sodium chloride concentration was 0.15 M. The activation energies (E_a) were 64.5 and 103 kJ mol^{-1}, respectively, for conc PME and Iso4 PME. Guava PME, cv Paluma, is a very thermostable enzyme (Leite *et al.*, 2006).

Thermal stability and kinetics behavior of hawthorn PME were evaluated by Vivar-Vera *et al.* (2007). PME extract showed maximum activity at 0.4 moles/L NaCl, pH 7.5, and 55°C. The Ea and Q_{10} for thermal activation were 36.27 kJ mol^{-1} and 2.01 (20–30°C), respectively. About 50% of the activity remained after heating for 25 min at 60°C, and it was completely inactivated by treatment at 80°C for 10 min. The Q_{10} and Ea values for the thermal inactivation reaction at 70–80°C were 20.06 and 146.16 kJ mol^{-1}, respectively.

Studies on the thermal inactivation of apple and mango PME are scanty. Also, there are no published data on the thermal inactivation of cauliflower PME. Therefore, the scope of the present work is aimed to evaluate the thermal stability and kinetic behavior of PME in apple, mango and cauliflower to optimize the process aiming to stabilize their products.

MATERIALS AND METHODS

Materials:

Apple (*Malus domestica* var. Anna) fruits at the ripe stage were obtained from Ismailia local market. Mango (*Mangifera indica* var. Zebda) fruits at mature stage (allowed to ripen at room temperature) were picked from a private garden in Ismailia governorate. Cauliflower (*Brassica oleracea* var. Botrytis) heads were purchased from Ismailia local market.

Chemicals:

All fine chemicals used in the determination of the enzyme activities were obtained from Sigma-Aldrich chemical company, while the other chemicals used were of analytical grade.

Methods:

Preparation of PME crude extracts:

PME was extracted from the three examined fruits (apple, mango and cauliflower) using the procedures of Abu-Goukh and Bashir (2003). Cauliflower heads were washed, trimmed and prepared as small florets. Mango fruits were washed, peeled and de-seeded. The apples were washed, peeled, diced into 1 cm cubes. From each the tested samples (200 g) were homogenized in an equal volume of 100 mM sodium acetate buffer, pH 6.0 containing sodium dithionite 0.2 % ($Na_2S_2O_4$) and polyvinyl pyrrolidone (1 %) for 1 min using a blender (Matsushita ELEC. IND. CO., LTD. Japan). The homogenates were centrifuged at 12,000 xg for 20 min (supernatant 1).The residues were suspended in an equal volume of 1 M sodium acetate buffer containing NaCl 6 % (pH 6.0). The pH of the three suspensions was adjusted to 6.5 for mango and to 6.0 for cauliflower and apple, using drops of NaOH (2N). The suspensions were kept at 4°C with continuous stirring for 4 hrs and then centrifuged. The supernatant for each sample was filtered twice using Whatman filter paper No 1 (supernatant 2). Mango pectin methyl esterase activity was determined in supernatant 1; while apple and cauliflower pectin methyl esterase activities were determined in supernatant 2.

PME assay:

PME activity was measured in the extracted samples according to the method described by Jiang *et al.* (2003). Reactions were started by the addition of 1 ml of cauliflower, apple or 2 ml of mango extracts to 15 ml of NaCl (0.1M) containing citrus pectin solution (0.5 %) at 30°C. The pH of the reaction mixtures was adjusted at 7.5 for apple and mango and to 8.0 for cauliflower immediately before assay. The activity of PME was followed with a pH meter (model 3305 JENWAY, U.K.) by titrating the free protons, which were dissociated from the free carboxyl groups that had been formed by the PME activity. The volumes (ml) of 0.01 N NaOH consumed to maintain the pH at 7.5 for apple and mango and at 8.0 for cauliflower were recorded within a reaction time of 10 min. An enzyme solution previously heated in boiling water for 5 min was used as a blank.

Calculation:

The PME activity was expressed in units (μmoles of H^+ produced per min) according to the following equation:

$$\text{Unit (U)} = \frac{(Vs-Vb) \times M_{NaOH} \times 1000}{V \times t}$$

Where:

- Vs = NaOH used to titrate the sample (ml),
- Vb = NaOH used to titrate the blank (ml),
- M_{NaOH} = NaOH concentration (molarity),
- V = sample (ml),
- t = time of analysis.

Heat inactivation:

Aliquots of the extracts were transferred to capillary tubes of 2.6 mm internal diameter and 100 mm length with a syringe. The tubes were heated in a circulating water bath (BUCHI 462. Switzerland) to temperatures adjusted between 50 to 95°C (±1°C). After preset times and temperatures, the tubes were removed successively from the water bath and placed in ice to stop thermal inactivation. The residual activities of the enzymes were assayed without any further treatments as described previously.

RESULTS AND DISCUSSION

Heat inactivation curves of the crude PME from apple, mango and cauliflower are shown in Fig. (1). The inactivation rate constants increased with increasing temperature. For example, the apple PME (Fig. 1-A) was reasonably stable at 50 and 55°C, but upon exposure to 60°C there was 50% residual activity after 600 S. At 65°C, only 20% of the residual activity was detected after 500 S. While, at 70°C, ~ 90% of the activity was lost after 100 S. On the other hand, the mango PME (Fig. 1-B) was reasonably stable at 50 to 65°C and when exposed to 70°C, the residual activity was 40% after 600 S. This ratio decreased to 16 % at 75°C after the same time. While, at 80 and 85°C, there were 12 % residual activities after 240 and 180 S., respectively. At 90°C, ~ 90% of the activity was lost after 60 S. Fig. (1-C) showed that the PME activity of cauliflower was stable at 50°C and when exposed to 55°C, the residual activity was 50% after 300 S. While, at 60°C, the enzyme activity lost about 90 % after 600 S. There was 6 % residual activity after 175 S. exposure at 65°C. While at 70 and 75°C, ~ 1.5 % residual activities were detected after 250 and 125 S., respectively.

Furthermore, the semilog plots of the thermal inactivation for both apple and mango PME (Fig. 1, A-B) were linear in accordance with the first order monophasic kinetics. These results are in agreement with those obtained by Denes *et al.* (2000) who found that the thermal inactivation of apple PME followed the first order denaturation process. Similarly, Labib *et al.* (1995) reported similar results for mango PME.

However, the semilog plot for cauliflower PME was nonlinear (biphasic) at temperatures of 55 to 65°C (Fig. 1-C). This indicates the presence of heat-labile and heat-resistant forms in the crude cauliflower PME extract. Such nonlinear heat inactivation was observed by Versteeg *et al.* (1980) for Navel orange PME crude extract. Also, Ly-Nguyen *et al.* (2002) found that the thermal inactivation curve of strawberry PME was nonlinear.

From the slopes of the inactivation lines for the three PME of the tested samples (Fig. 1), rate constants were calculated and plotted in Arrhenius plots (Fig. 2). The Arrhenius plots for both apple and cauliflower showed distinct upward curvature. At temperatures >55°C (i.e., at $1/T<0.00304$) the plots can be approximated by straight lines. On the contrary, the Arrhenius plot for mango PME was linear at all studied temperatures. From the slopes of these lines activation energies (Ea) were estimated. Also, Fig. (2) clears that mango PME enzyme was inherently more thermally stable than cauliflower than apple PME. A simple linear Arrhenius plot has been reported for tomato PME

(Anthon *et al*., 2002), but for carrots and potatoes PME show some deviation from linearity (Anthon and Barrett, 2002).

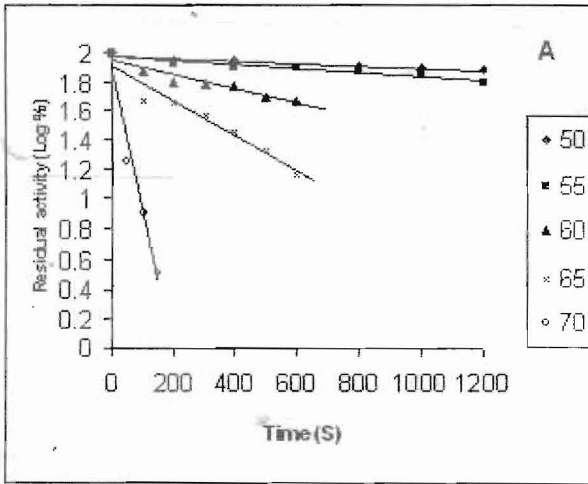
Table (1) reports D, z and Ea values (thermal resistance parameters) for the three PME extracts obtained with k values from Fig. (1). As expected, the inactivation rate constants increased with increasing temperatures. The data showed that thermal inactivation of apple, mango and cauliflower PME was comparable to that of orange (Van den Broeck *et al*., 2000) and strawberry (Ly-Nguyen *et al*., 2002).

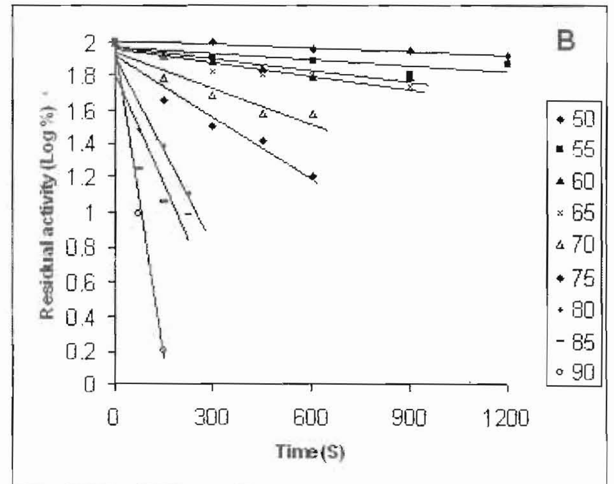
The obtained rate constants for the three extracts at 70°C were 22.11, 1.61 and 16.8 $\times 10^3$ S^{-1} for apple, mango and cauliflower PME, respectively. This means that mango PME was the most heat-stable, while apple PME was the lowest one.

The D value, the time of heating at a constant temperature required to inactivate 90% of the original enzyme activity, was calculated (Table 1). At 70°C the values were 1.7, 23.8 and 2.3 min. for apple, mango and cauliflower, respectively. The data showed that mango PME was more heat-stable than cauliflower than apple PME. Denes *et al*. (2002) reported a far lower D value of 0.2 min for apple PME at 65°C, which less than the results obtained (13.8 min.) probably due to apple variety. Labib *et al*. (1995) found that D value was 4.5 min. for mango PME at 80°C, this finding confirmed the fore mentioned pattern (4.5 min. at 80°C).

Z value was the temperature increase necessary to obtain a 10-fold decrease of the D value. The values were 7.91, 17.67 and 10.64°C for apple, mango and cauliflower, respectively as seen in Table (1). The results are in agreement with those reported by Denes *et al*. (2002) who investigated a z value of 9.2°C for apple PME. Also, Labib *et al*. (1995) reported a z value of 18.5°C for mango PME.

Thermal dependence of the rate constants, expressed as activation energy (Ea) for apple, mango and cauliflower PME was estimated (Table 1). The values were 272.24, 126.7 and 208.42 kJ mol^{-1} for apple, mango and cauliflower, respectively. These activation energies are higher than those reported by Denes *et al*. (2000) for apple PME 31.26 kJ mol^{-1}. But, they were lower than those reported for CXD and BOX tomato cultivars 477 and 440 kJ mol^{-1}, respectively (Anthon *et al*., 2002). The differences in Ea values could be due to differences in the raw materials or differences between crude and purified enzyme.





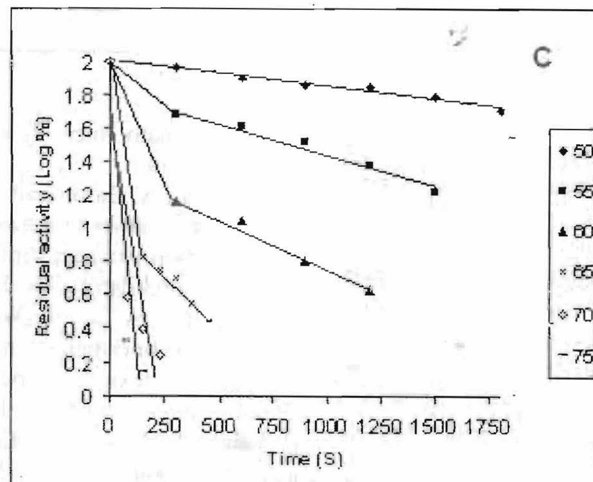


Fig. (1): Thermal inactivation of PME in apple (A), mango (B) and cauliflower (C).

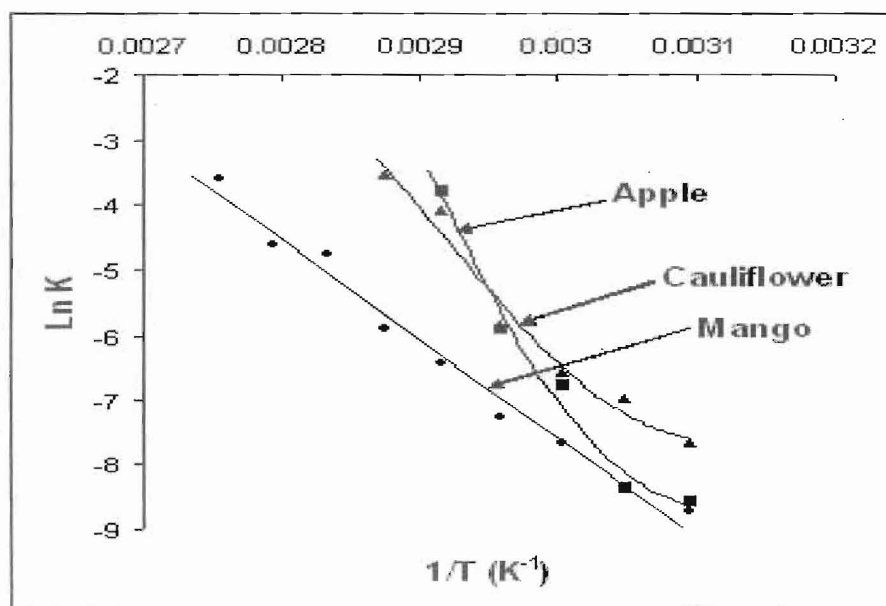


Fig. (2): Arrhenius plot of inactivation rates for PME from apple, mango and cauliflower.

Table (1): Parameters of first order model for PME from apple, mango and cauliflower.

Enzyme	Temp. (°C)	k $(x10^3)(S^{-1})$	R^2	D value (min)	z value (°C)	Ea $(kJ\ mol^{-1})$
Apple PME	50	0.18	0.914	208.3	7.91	272.24
	55	0.23	0.931	166.6		
	60	1.15	0.924	33.3		
	65	2.76	0.943	13.8		
	70	22.11	0.965	1.7		
Mango PME	50	0.16	0.928	238.1	17.67	126.7
	55	0.23	0.662	166.7		
	60	0.46	0.876	83.3		
	65	0.69	0.868	55.5		
	70	1.61	0.894	23.8		
	75	2.76	0.951	13.9		
	80	8.52	0.922	4.5		
	85	9.90	0.809	3.8		
	90	27.6	0.994	1.4		
Cauliflower PME	50	0.46	0.969	83.3	10.64	208.42
	55	0.92	0.974	41.7		
	60	1.38	0.984	27.8		
	65	2.99	0.977	12.8		
	70	16.81	0.76	2.3		
	75	28.55	0.822	1.3		

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حركيات التثبيط الحراري لإنزيم بكتين ميثيل استيريز في التفاح والمانجو و القنبيط

زكريا احمد صالح الشامي- أمال عبد الفتاح جاب الله- عادل ابوبكرشطا- أحمد محمد محمد ريان

قسم الصناعات الغذائية- كلية الزراعة- جامعة قناة السويس- ٤١٥٢٢ الإسماعيلية- مصر

قدرت حركيات التثبيط الحراري لإنزيم بكتين ميثيل إستيريز المستخلص من التفاح والمانجو و القنبيط علي درجات حرارة تراوحت بين ٥٠، ٩٠°م.إتبعت معدلات التثبيط الحراري حركيات الرتبة الأ- ولي. علي درجة حرارة ٧٠°م كانت قيم زمن التخفيض العشري ١,٧و ٢٣,٨و٢,٣ دقيقة و قيم مقياس المقاومة للحرارة ٧,٩١ و١٧,٦٧ و١٠,٦٤°م وقيم طاقة التنشيط ٢٧٢,٢٤ و ١٢٦,٧و ٢٠٨,٤٢ كيلو جول مول-للتفاح والمانجو والقنبيط علي الترتيب وأوضحت النتائج أن إنزيم بكتين ميثيل ا ستيريز المانجو كان أكثرهم ثباتا للحرارة بينما كان إنزيم التفاح اقلهم ثباتا.