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EFFECT OF CALCIUM AND SUCROSE ON THE THERMAL INACTIVATION OF PECTIN METHYLESTERASE FROM TOMATO FRUITS: A KINETIC STUDY

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ABSTRACT

Background. The aim of this study was to investigate the effect of calcium and sucrose, which are in several cases present in fruit-based foods during food processing, on the thermal inactivation kinetics of pectin methylesterase extracted from tomato fruits.

Material and methods. Pectin methylesterase from tomato fruits was extracted using 0.2 M KH_2PO_4/K_2HPO_4 buffer containing 1.0 M NaCl (pH 8.0) (1:1 w/v). The activity of pectin methylesterase was determined using an automatic pH-Stat. The thermal inactivation kinetics of the crude tomato pectin methylesterase extract were studied in the absence or presence of calcium (50 mM) or sucrose (20%) in a temperature range of 60°C to 69°C and calculated using non-linear regression analysis (using SAS 9.1 software).

Results. The thermal inactivation of tomato pectin methylesterase followed a fractional-conversion kinetic model, indicating the presence of a first-order inactivating thermal sensitive PME fraction and the occurrence of a thermostable PME fraction. Tomato pectin methylesterase is more stable in thermal treatment compared to pectin methylesterase extracted from other fruits. Calcium and sucrose retarded the inactivation of pectin methylesterase but did not change the behavior of the enzyme during thermal treatments.

Conclusion. Calcium and sucrose had protective effects on pectin methylesterase activity during thermal treatments. This finding is a good point of reference for food processors working in the area of fruit juice processing.

Keywords: activation energy, fractional-conversion model, PME, protective effect, thermal inactivation, thermal stable fraction

INTRODUCTION

Pectin methylesterase (PME) catalyzes the de-esterification of a polygalacturonic acid polymer, leading to the formation of acidic pectin which can cross-link with Ca^{2+} ions to form a precipitate of calcium pectate (Alonso et al., 1995; Ben-Shalom et al., 1985; Powell et al., 1982; Walkinshaw and Arnott, 1981). As a consequence, the action of PME can cause cloud loss (phase separation) of fruit and vegetable juices during processing and storage (Laratta et al., 1995; Rombouts et al., 1992).

Recently, tomato juice and tomato-based juices have taken up a large market share in the global fruit and vegetable industry. Naturally, tomato juices are characterized by a suspension colloid state, forming sensory characteristics that are typical for tomato-based products. Unfortunately, this suspension colloid

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state is easily broken as a result of the consecutive action of pectin methylesterase and polygalacturonase. To deal with this situation, food processors conduct thermal treatments to inactivate PME. This action is intended to conserve suspension colloid systems.

Next to enzyme structure (e.g. amino acid sequence, number of salt bridges, hydrogen bonds, disulphide bonds and hydrophobic interactions), micro-environmental conditions such as water content, pH, and the presence of salts or sugars also affect enzyme stability (Volkin et al., 1991; De Cordt et al., 1994). Van den Broeck et al. (1999b) reported that calcium ions accelerate the thermal inactivation of orange PME, whereas the temperature sensitivity of the inactivation rate constant decreased. Increasing the molarity of the calcium chloride solution increased inactivation but decreased the activation energy. This could indicate that PME is inactivated more quickly in natural products in which calcium ions are naturally present.

It is also worth mentioning that a lower initial activity of PME was observed in the presence of calcium ions. The lowest initial activity of PME was observed at the highest concentration of calcium ions. Alonso et al. (1997) studied the effect of calcium ions on PME isoforms of persimmon fruits and reported that both isoforms had optimum activity at calcium chloride concentrations between 60 mM and 70 mM. Above these concentrations, the inhibition of PME occurred. According to Nari et al. (1991), the activation of PME by metallic ions appeared to be mainly due to the interaction of ions with the substrate rather than with the enzyme. The metallic ions would release the enzyme molecules trapped in the blocks of free carboxyl groups, enabling them to reactivate.

Sucrose has a protective effect on the activity of PME, as it acts as a non-competitive inhibitor of PME. Sucrose stabilises the structure of the enzyme and increases its thermal resistance (Chang et al., 1964). At a natural concentration (4%) in juice, sucrose stabilizes PME to a small extent, while at a higher concentration (20%) sucrose clearly slows down the inactivation. An environment of lower water activity due to sucrose may be an important factor involved, since the denaturation of enzymes requires water. Adding sucrose only slightly influences the activation energy (Van den Broeck et al., 1999b). The addition of sucrose (500 mg/mL) and trehalose (500 mg/mL) to purified tomato PME resulted in a marked increase in thermostability, as indicated by the increase in D-values; however, no significant change in the z-value was observed (Guiavarc'h et al., 2003). This study aimed to investigate the effect of calcium and sucrose on the thermal stability of crude tomato PME.

MATERIAL AND METHODS

Material and chemical

The tomato fruits (*Lycopersicon esculentum*, cv Arka F1) were purchased from local markets (Can Tho city, Vietnam), and apple pectin (DE 75%) from Sigma-Aldrich (Buchs, Switzerland). All other chemicals were of analytical grade and were purchased from local suppliers.

PME extraction

The extraction of tomato PME was performed according to the method of Wicker (1992), which is presented in Figure 1. Materials were homogenized in batches of 1.0 kg with the addition of de-ionised water (2:1 w/v) using a blend mixer. The pellets were collected by centrifugation at 10,000×g for 30 min. The pellets were then washed with de-ionised water (2:1 w/v; w is based on the weight of the raw materials) and collected again by centrifugation/filtration. The washing step was repeated twice. The supernatants were discarded, while the washed pellets were mixed overnight at 4°C in 0.2 M KH_2PO_4/ K_2HPO_4 buffer containing 1.0 M NaCl (pH 8.0) (1:1 w/v) for the extraction of PME bound to the cell wall. Subsequently, the salt extracts, collected by centrifugation at 10,000×g for 60 min, were fractionized, first by ammonium sulfate precipitation at 30% saturation while being continuously stirred at 4°C for 30 min, followed by centrifugation at 18,000×g for 15 min at 4°C to remove contaminating proteins. After filtering off the pellets of the contaminating proteins, a second precipitation was carried out in 80% ammonium sulphate saturation, stirring at 4°C for 30 min, and then centrifugating at 18,000×g for 15 min. The crude PME was collected as precipitates and dissolved in 20 mM KH_2PO_4/ K_2HPO_4 buffer (pH 7.5), while the supernatants in the second case were discarded. The amount of buffer used for the dissolution corresponded to 5 mL per 100 g of fresh materials. The crude tomato PME collected was used for further experiments.

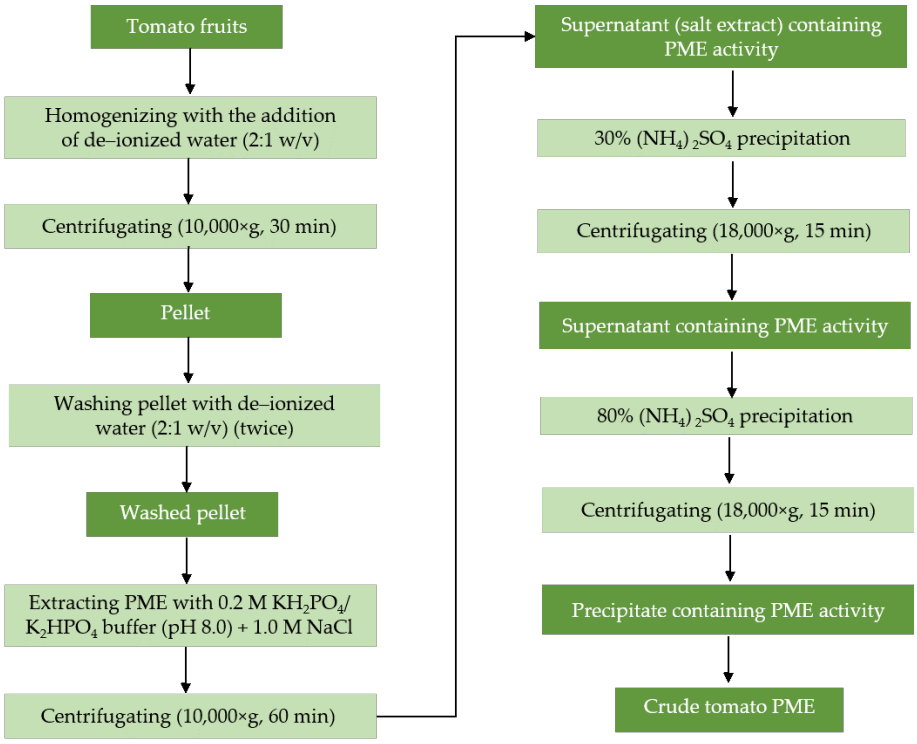

Fig. 1. Crude tomato PME extraction scheme

PME assay

As PME action results in the removal of methoxyl groups from pectin and the production of methanol and H^+ ions, the enzyme activity can be followed continuously by titration (Giovane et al., 1996). In the course of this study, PME activity was determined by continuous recording of the titration of the carboxyl groups released from a pectin solution with 0.01 N NaOH using an automatic pH-Stat (Metrohm, Herisau, Switzerland). Routine assays were performed with a 3.5 mg mL^{-1} pectin solution (DE 75%, 30 mL) containing 0.117 M NaCl at pH 7.0 and 22.5°C. One unit of PME is defined as the amount of enzyme required to release 1 µmol of carboxyl groups per minute, under the aforementioned conditions (Reed, 1966). PME activity measured in units/mL (U/mL) is therefore computed by the following formula:

$$\text{PME activity (Units/mL)} = = \frac{[\text{Vol of NaOH (mL)}][\text{Molarity of NaOH (M)}]10^6}{[\text{Time (min)}][\text{Vol of sample (mL)}]10^3} \quad (1)$$

Since the titration yields the PME activity of a given sample size in mL of NaOH used per minute, and the molarity of NaOH is known (0.01 M), the PME activity was then computed by the following simplified formula:

$$\text{PME activity (Units/mL)} = = \frac{[\text{Titration reading (mL/min)}]10}{[\text{Vol of sample (mL)}]} \quad (2)$$

Thermal inactivation of crude tomato PME

The crude tomato PME was thermally treated in the absence or presence of calcium (50 mM) or sucrose (20%) within a temperature range of 60°C to 69°C. Isothermal treatments were performed in a temperature-controlled water bath using glass tubes to enclose the enzyme solution. After treatments, the tubes were immediately cooled in ice water. The residual activities of PME were measured within 60 min of storage at 0°C using acid-base titration (Titrino 785, Metrohm AG, Herisau, Switzerland). The thermal inactivation

kinetics of the enzyme were calculated (non-linear regression analysis, SAS 9.1 package).

Kinetic data analysis

As previously published by Van den Broeck et al. (1999a), Ly Nguyen et al. (2002a; 2002b), Castro et al. (2006), and Ando et al. (2017), the inactivation of plant PME followed a fractional-conversion model. This model applies when the enzyme sample contains a stable fraction that is not affected under the processing conditions studied (Eq. 3).

$$A = A_\infty + (A_0 - A_\infty)\exp(-kt) \quad (3)$$

where A_0 and A are, respectively, the initial activity and the remaining activity at time t (min); A_∞ is the remaining activity after prolonged treatment; and k is the inactivation rate constant (min^{-1}).

It should be stressed that for the experiments at a constant temperature, the heating time should be long enough so that the remaining activity A_∞ is no longer changing with respect to time (Van den Broeck et al., 1999a; 1999b).

Once the inactivation rate constants k at different temperatures are known, the activation energy (E_a) of enzyme thermal inactivation can be estimated using the Arrhenius relationship (Eq. 4).

$$\ln k = \ln k_{ref} + \left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \quad (4)$$

where k_{ref} is the inactivation rate constant at the reference temperature (min^{-1}), R is the universal gas constant (8.3144 J mol^{-1} K^{-1}), T is the absolute temperature (K), and T_{ref} is the absolute reference temperature (K).

The activation energy can be estimated by linear regression analysis of the logarithm of the rate constant versus the inverse of absolute temperature.

Next to two-step regression analysis, one-step regression analysis can also be used to predict the kinetic parameters (non-linear regression). In this case, the inactivation/degradation data obtained for different treatment temperatures are simultaneously considered and the inactivation rate constant is determined at a reference temperature. The mathematical model of this case is presented below (Eq. 5).

$$A = A_\infty + (A_o - A_\infty)\exp\left(-k_{ref}\exp\left(\frac{E_a}{R}\left(\left(\frac{1}{T_{ref}}\right) - \left(\frac{1}{T}\right)\right)\right)t\right) \quad (5)$$

The accuracy of the regression equations was evaluated by the calculation of corrected R^2 (Eq. 6) and standard deviation (Eq. 7) of each estimated model parameter (Indrawati et al., 2001).

$$\text{Corrected.}R^2 = \left[1 - \frac{(m-1)\left(1 - \frac{SSQ_{Model}}{SSQ_{CorrectedTotal}}\right)}{(m-j)}\right] \quad (6)$$

$$SD = \sqrt{\frac{SSQ_{Error}}{(m-j)}} \quad (7)$$

where m is the number of observations; j is the number of model parameters; SSQ_{Model} is the sum of squares of 'Model'; $SSQ_{CorrectedTotal}$ is the sum of squares of 'Corrected Total'; SSQ_{Error} is the sum of squares of 'Error'; and SD is standard deviation.

Enthalpy and free energy were described by Eyring models (Eqs. 8 and 9). Enthalpy at each temperature was calculated according to Eq. 8.

$$\Delta H = E_a - RT \quad (8)$$

where ΔH represents enthalpy (kJ mol^{-1}) and E_a is activation energy (kJ mol^{-1}).

Free energy (kJ mol^{-1}) at different temperatures was calculated by Eq. 9.

$$\Delta G = -RT\ln(kh/(k_B.T) \quad (9)$$

where k is the inactivation constant rate of PME, h is the Planck constant (6.6262×10^{-34} J) and k_B is the Boltzmann constant (1.3806×10^{-23} J K^{-1}).

Statistical analysis

All experiments were performed in triplicate and the presented results are the mean of the experimental data. Analyses of variance (ANOVA) with a significance level of $p < 0.05$ were performed. All statistical analyses were conducted using the SAS package 9.1.

RESULTS AND DISCUSSION

Thermal inactivation kinetics of crude tomato PME

The isothermal inactivation, at atmospheric pressure, of crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5) could be accurately described by a fractional-conversion kinetic model (Eqs. 3 and 5) in the temperature range of 60°C to 67°C, indicating the presence of a first-order inactivating thermal sensitive tomato PME fraction and the occurrence of a thermostable tomato PME fraction (Fig. 2). Inactivation rate constants and thermostable tomato PME fractions at different temperatures, estimated using non–linear regression analysis of Eqs. 3 and 5, are presented in Table 1. As expected, the inactivation rate constants increase with increasing temperatures. The crude tomato PME is more stable to thermal treatment compared to other plant PMEs. About 10.2–21.0% of the total activity was attributed to the thermostable tomato PME fraction as estimated using two-step regression analysis, while it contributed 12.7% of the total activity as calculated using one-step regression analysis. For the crude tomato PME (pH 7.5), as the treatment temperatures increased from 60°C to 67°C, inactivation rate constants ranging from 0.0287 to 0.4261 min^{-1} were obtained. As reported by Fachin et al. (2002), similar thermal inactivation rate constants were observed for tomato PME inactivation (in juice) (data not shown). Van den Broeck et al. (1999a; 1999b) obtained inactivation rate constants between 0.103 and 0.165 min^{-1} at 60°C for commercial orange PME and between 0.055 and 0.112 min^{-1} for crude orange PME inactivation, both thermally treated in water. The same authors (Van den Broeck et al., 1999b) reported inactivation rate constants between 0.035 and 0.174 min^{-1} at 60°C for crude orange PME when the enzyme was thermally treated in citric acid buffers in a pH range of 3.2–4.2.

Thermal inactivation kinetics of crude tomato PME in the presence of calcium

The isothermal inactivation of crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5) in the presence of calcium could also be described by a fractional-conversion kinetic model in the temperature range of 63°C to 67°C (Fig. 3). Inactivation rate constants and thermostable fractions of the crude tomato PME at different temperatures estimated using non-linear regression analysis are presented in Table 2. From the data shown in Table 2, it is apparent

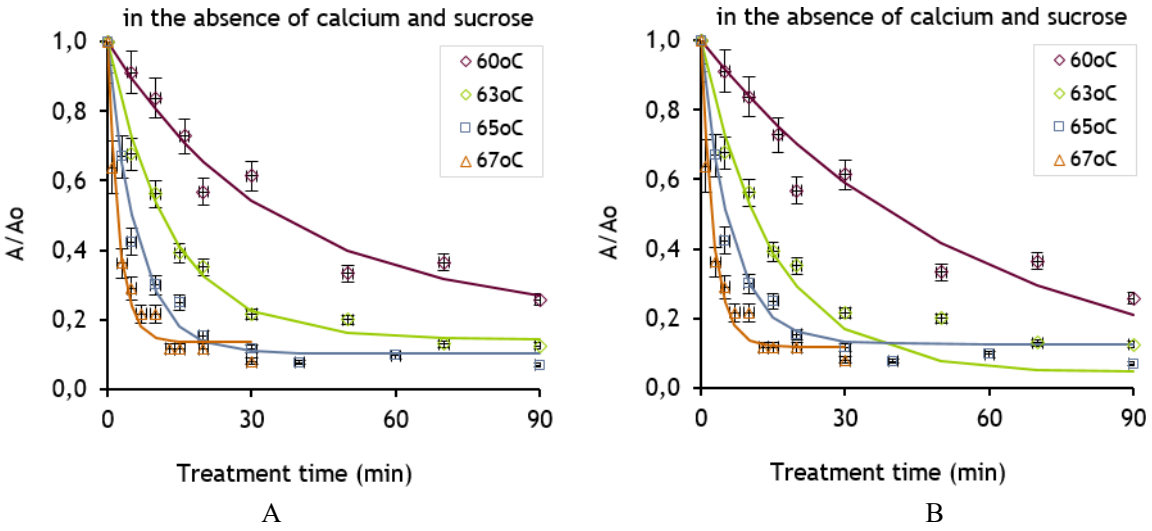

Fig. 2. Fractional-conversion thermal inactivation of crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5) in the absence of calcium and sucrose. Symbols represent experimental data points; full lines represent the best fit based on non-linear regression analysis using (A) the two-step approach and (B) the one-step approach

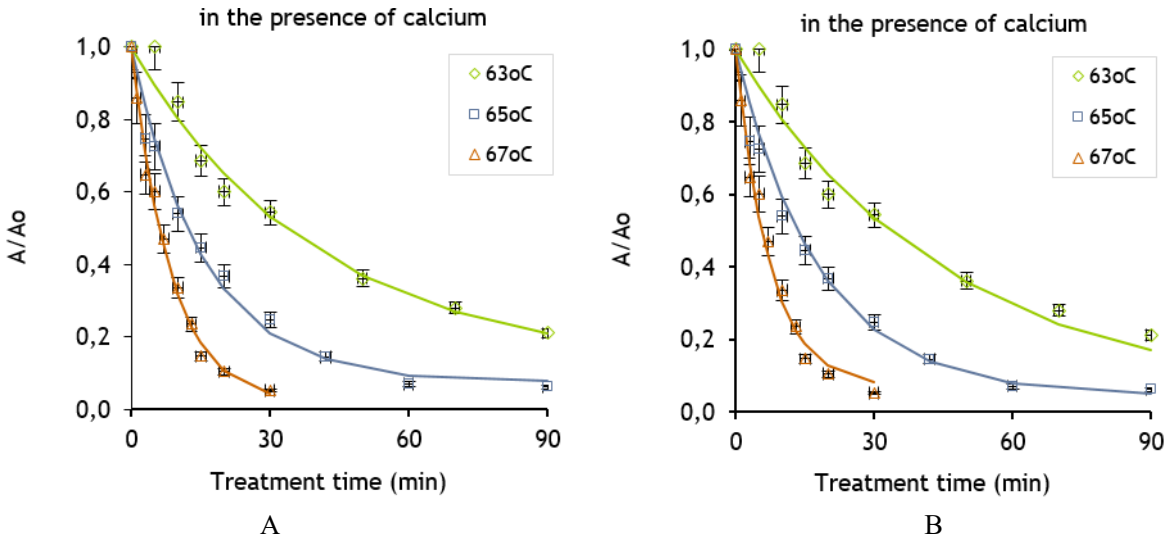

Fig. 3. Fractional-conversion thermal inactivation of crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5) in the presence of calcium. Symbols represent experimental data points; full lines represent the best fit based on non-linear regression analysis using (A) the two-step approach and (B) the one-step approach

that when adding calcium to the PME samples the inactivation rate constant presented smaller values than were obtained when calcium was not added for each corresponding treatment temperature (see Table 1). A similar trend was reported by Nari et al. (1991) in a study on the interaction of metallic ions with plant

Table 1. Kinetic parameter estimates of a fractional-conversion model for isothermal inactivation of crude tomato PME (in the absence of calcium and sucrose)

Two-step regression analysis approach				
Temperature, °C	k, min^{-1}	A_∞, %	Corrected R^2	SD
60	0.0287 ±0.0059[a]	21.0 ±7.4	0.994	0.0549
63	0.0781 ±0.0053	14.4 ±1.8	0.997	0.0302
65	0.1622 ±0.0147	10.2 ±1.9	0.992	0.0414
67	0.4261 ±0.0510	13.5 ±2.1	0.989	0.0468
E_a = 358.3 ±24.2 kJ/mol				
One-step regression analysis approach				
60	0.0181 ±0.0012	12.7 ±1.8	0.989	0.0553
63	0.0680 ±0.0034			
65	0.1625 ±0.0112			
67	0.3844 ±0.0379			
E_a = 411.6 ±17.6 kJ/mol				

[a]Standard error of regression.

Table 2. Kinetic parameter estimates of a fractional-conversion model for isothermal inactivation of crude tomato PME (in the presence of calcium)

Two-step regression analysis approach				
Temperature, °C	k, min^{-1}	A_{∞}, %	Corrected R^2	SD
63	0.0253 ±0.0047[a]	12.1 ±7.9	0.995	0.0493
65	0.0638 ±0.0056	7.5 ±2.8	0.995	0.0406
67	0.1174 ±0.0097	1.5 ±3.4	0.997	0.0329
E_a = 364.9 ±42.0 kJ/mol				
One-step regression analysis approach				
63	0.0220 ±0.0013	4.4 ±2.0	0.995	0.0436
65	0.0549 ±0.0033			
67	0.1354 ±0.0117			
E_a = 431.3 ±21.4 kJ/mol				

[a]Standard error of regression.

pectin methylesterase and its substrate. According to Nari et al. (1991), the activation of PME by metallic ions appeared to be due mainly to the interaction of ions with the substrate rather than with the enzyme. The metallic ions release the enzyme molecules trapped in the blocks of free carboxyl groups, enabling them to reactivate.

Thermal inactivation kinetics of crude tomato PME in the presence of sucrose

Similarly, the isothermal inactivation of crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5) with the presence of sucrose could be described by a fractional-conversion kinetic model in the temperature range of 63 to 69°C (Fig. 4). Inactivation rate constants and thermostable fractions of the crude tomato PME at different temperatures estimated using non-linear regression analysis are presented in Table 3. From the data shown in Table 3, it is apparent that the inactivation rate constants were smaller than those obtained in the absence of calcium and sucrose for each corresponding treatment temperature. Sucrose also had a protective effect on the activity of PME against thermal treatment. Sucrose has a protective effect on the activity of PME because it acts as a noncompetitive inhibitor of PME by occupying the active site. It stabilizes the structure of the enzyme and increases its thermal resistance (Wilińska et al., 2008). At the natural concentration (4%) found in juice, sucrose stabilizes PME to a small extent, whereas at a higher concentration (20%) sucrose clearly slows down the inactivation. An environment of lower water activity due to sucrose may be an important factor here, since the denaturation of enzymes requires water.

The temperature dependence of the inactivation rate constants estimated in the temperature ranges studied (i) in the absence of calcium and sucrose (Table 1), (ii) in the presence of calcium (Table 2), and (iii) in the presence of sucrose (Table 3) could be adequately described by the Arrhenius equation (Eq. 4) (Fig. 5), producing activation energy values of 358.3, 364.9, and 364.0 kJ/mol, respectively, as estimated using two-step regression analysis, and 411.6, 431.3, and 502.2 kJ/mol, respectively, as estimated using one-step regression analysis. These observations are comparable to those obtained by Van den Broeck et al. (1999a, 1999b) for the thermal inactivation of commercial orange PME, by Massaguer et al. (1994) for the thermal inactivation of heat-labile and heat-stable PME fraction from acidified papaya pulp, and by Fachin (2003) for the thermal inactivation of tomato PME in crude extract, purified form, and juice, as reported in Table 4.

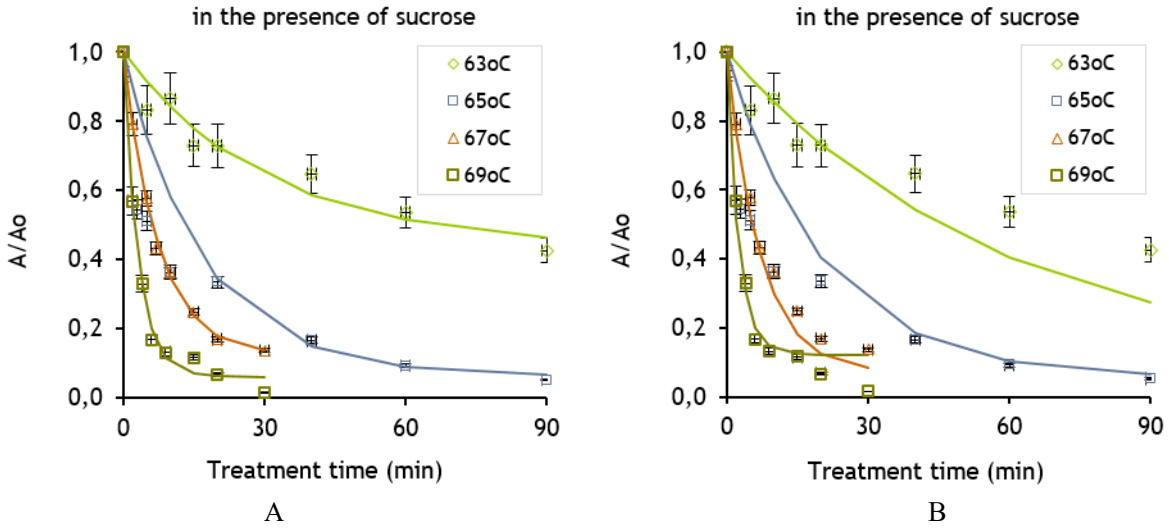

Fig. 4. Fractional-conversion thermal inactivation of crude tomato PME dissolved in 20 mM KH_2PO_4/ K_2HPO_4 buffer (pH 7.5) in the presence of sucrose. Symbols represent experimental data points; full lines represent the best fit based on non-linear regression analysis using (A) the two-step approach and (B) the one-step approach

The activation energy of the thermal inactivation of an enzyme is closely related to the inactivation rate. High activation energy reveals the high thermal resistance of PME (Aghajanzadeh et al., 2016). The higher the activation energy, the slower the inactivation. Activation energy reflects the amount of energy required to

Table 3. Kinetic parameter estimates of a fractional-conversion model for isothermal inactivation of crude tomato PME (in the presence of sucrose)

Two-step regression analysis approach				
Temperature, °C	k, min^{-1}	A_{∞}, %	Corrected R^2	SD
63	0.0330 ±0.0084[a]	43.6 ±6.3	0.996	0.0508
65	0.0597 ±0.0032	6.1 ±1.2	0.999	0.0106
67	0.1364 ±0.0056	12.2 ±1.3	0.999	0.0148
69	0.3174 ±0.0229	6.1 ±1.7	0.995	0.0311
E_a = 364.0 ±21.6 kJ/mol				
One-step regression analysis approach				
63	0.0172 ±0.0027	5.3 ±2.3	0.962	0.1115
65	0.0497 ±0.0059			
67	0.1421 ±0.0205			
69	0.4011 ±0.0842			
E_a = 502.2 ±43.8 kJ/mol				

[a]Standard error of regression.

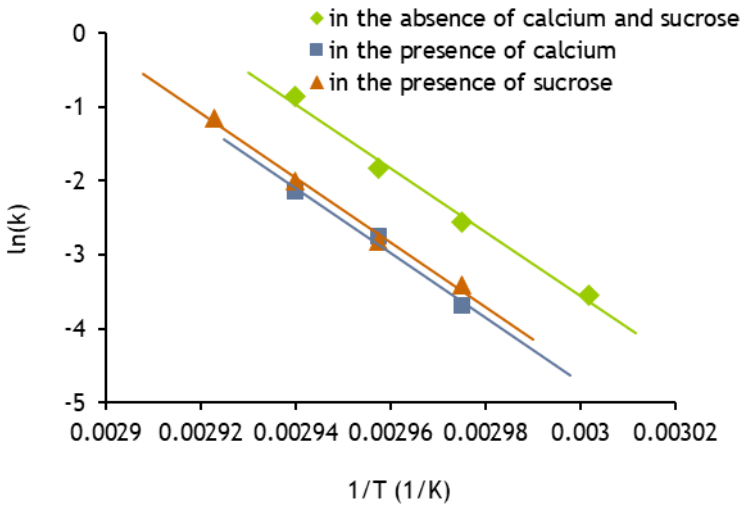

Fig. 5. Temperature dependence of inactivation rate constants for the thermal inactivation of crude tomato PME

Table 4. The activation energy for the thermal inactivation of PME from different sources

Source of PME	E_a, kJ/mol	Reference
Crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5), in the presence of sucrose at a level of 20%	502.2 ±43.8^{A*}	own observation, by one-step regression analysis approach
Purified tomato PME dissolved in 50 mM citrate buffer (pH 4.4)	483.4±28.9^{AB}	Fachin (2003)
Crude tomato PME dissolved in 50 mM citrate buffer (pH 4.4)	479.7±17.8^{ABC}	Fachin (2003)
Crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5), in the presence of calcium	431.3 ±21.4^{BCD}	own observation, by one-step regression analysis approach
Crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5), in the absence of calcium and sucrose	411.6 ±17.6^{CDE}	own observation, by one-step regression analysis approach
Crude orange thermolabile PME fraction dissolved in water (cv Valencia)	372.8±12.7^{DEF}	Van den Broeck et al. (1999b)
Crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5), in the presence of calcium	364.9 ±42.0^{DEF}	own observation, by two-step regression analysis approach
Crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5), in the presence of sucrose	364.0 ±21.6^{DEF}	own observation, by two-step regression analysis approach
Tomato PME in juice (pH 4.4)	363.8 ±10.1^{DEF}	Fachin (2003)
Crude tomato PME dissolved in 20 mM KH_2PO_4/K_2HPO_4 buffer (pH 7.5), in the absence of calcium and sucrose	358.3 ±24.2^{DEF}	own observation, by two-step regression analysis approach
Commercial purified orange peel thermolabile PME fraction dissolved in water	350.5 ±22.8^{EF}	Van den Broeck et al. (1999a)
Crude orange PME dissolved in water (cv Navel)	344.1 ±15.2^{EF}	Van den Broeck et al. (1999b)
Thermostable PME fraction in acidified papaya pulp (pH 3.8)	303.9^{FG}	Massaguer et al. (1994)
Thermolabile PME fraction in acidified papaya pulp (pH 3.8)	257.9^{G}	Massaguer et al. (1994)
	$p < 0.0001$	

*Means with the same letter are not significantly different.

inactivate an enzyme. This index is more than just an energy barrier. It provides an energy threshold to be reached to inactivate an enzyme.

A significant difference test for the data reported in Table 4 was performed using SAS 9.1 (proc Anova). As observed in Table 4, the activation energy value for the thermal inactivation of crude tomato PME in the presence of sucrose at the level of 20% estimated by one-step regression analysis was highest and significantly different from those of other treatments except for the thermal inactivation of crude- and purified tomato PME dissolved in 50 mM citrate buffer (pH 4.4) reported by Fachin (2003). From this observation, it can be concluded that sucrose at high concentrations (i.e. 20%) and metallic ions (i.e. sodium citrate 50 mM buffer) are the two main factors among those increasing activation energy to higher levels. Sucrose present in the environment reduced the water activity of PME solution, while the denaturation or the inactivation of enzymes required water. To a certain extent, metallic ions activated PME as they interacted with substrate and released enzyme molecules trapped in the complex of enzyme-substrate.

Table 5. Thermodynamic parameters of the isothermal inactivation of crude tomato PME

Treatment	Temperature, °C	Enthalpy, kJ/mol	Free energy, kJ/mol
In the absence of $CaCl_2$ and sucrose	60	355.53 ±21.43	91.73 ±18.86
	63	355.51 ±21.41	89.79 ±6.09
	65	355.49 ±21.39	88.28 ±8.00
	67	355.47 ±21.37	86.09 ±10.30
In the presence of $CaCl_2$	63	362.11 ±39.21	92.94 ±17.27
	65	362.09 ±39.19	90.91 ±7.98
	67	362.07 ±39.17	89.74 ±7.41
In the presence of sucrose	63	357.21 ±18.81	92.20 ±23.47
	65	357.19 ±18.79	91.09 ±4.88
	67	357.17 ±18.77	89.31 ±3.67
	69	357.16 ±18.76	87.45 ±6.31
		($p > 0.05$)	($p > 0.05$)

Thermodynamic parameters (enthalpy and free energy) for the thermal inactivation of crude tomato PME with and without calcium and sucrose were estimated (Table 5). Statistically, the enthalpy values (ΔH) calculated for all thermal treatments decreased insignificantly ($p > 0.05$) as the temperature increased. As enthalpy is related to bond strength and is the measure of the energy obstacle that must be overcome by reacting molecules (Vikram et al., 2005), the insignificant variations in ΔH of all the treatments shown in Table 5 represent an insignificant change in PME structure during heat treatments. The positive value of enthalpy also indicates that the inactivation of crude tomato PME is an endothermic reaction (D'Amico et al., 2003; Nielsen et al., 2003). Free energy (ΔG) measures the spontaneity of a reaction (D'Amico et al., 2003). The positive value of free energy proves that the thermal inactivation of tomato PME is not a spontaneous reaction. The high value of ΔG in this study confirms the high thermal resistance of tomato PME (Aghajanzadeh et al., 2016). ΔG decreased insignificantly as temperature increased ($p > 0.05$).

CONCLUSIONS

The isothermal inactivation of the crude tomato PME followed the fractional-conversion kinetic model, indicating the presence of a first-order inactivating thermal-sensitive PME fraction and the occurrence of a thermostable PME fraction. For this stable PME fraction, high-pressure processing could be an alternative approach. Calcium stimulated tomato PME activity, while sucrose showed a 'protective effect' on PME activity but did not change the behavior of the enzyme during thermal processing.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

OPEN ACCESS

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