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Partial Purification, Characterization, and Thermal and High-Pressure Inactivation of Pectin Methylesterase from Carrots (*Daucus carrota* L.)

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Pectin methylesterase (PME) from carrots (*Daucus carrota* L.) was extracted and purified by affinity chromatography on a CNBr-Sepharose 4B-PME inhibitor column. A single protein and PME activity peak was obtained. A biochemical characterization in terms of molar mass (MM), isoelectric points (p*I*), and kinetic parameters of carrot PME was performed. In a second step, the thermal and high-pressure stability of the enzyme was studied. Isothermal and combined isothermal−isobaric inactivation of purified carrot PME could be described by a fractional-conversion model.

KEYWORDS: ***Daucus carrota* L.; carrots; pectin methylesterase; purification; thermal inactivation; high-pressure inactivation**

INTRODUCTION

Pectin methylesterase (pectinesterase, PME, PE, EC 3.1.1.11) is widely distributed in plants and microorganisms (*1*). PME catalyzes the de-esterification of methyl ester of polygalacturonic acid polymer to form pectic acid, methanol, and hydrogen ions, as part of an array of pectin hydrolyzing enzymes, and leads to the formation of a calcium pectate gel (*2*−*6*). Detrimental effects of PME on cloud stability of juices and nectars have been reported in detail (*7*−*11*). In contrast, beneficial effects of PME, including (i) enhancement of firmness of thermally processed fruit and vegetable products (*2*, *3*, *12*−*14*), (ii) effective increase of extraction yield of juices by conventional methods (*15*), and (iii) promotion of water removal from tissues on drying (*16*), have also been reported.

In plants, PME is bound to the cell wall by electrostatic interaction, and high-ionic-strength solutions are required in order to solubilize the enzyme and obtain a good yield of extraction (*17*−*25*). PME has been extracted and/or purified from different sources: e.g., tomatoes (*23*, *26*−*33*), oranges (*18*, *21*, *34*−*40*), apples (*17*, *24*, *41*−*46*), grapefruits (*22*, *47*, *48*), and bananas (*49*−*51*). Biochemical properties, thermal stability and food applications of PME from these sources were reported in more or less details by several authors.

PME from carrot (*Daucus carrota* L.) has been investigated by Polacsek and Pozsar (*52*), Markovic (*53*), Tijskens et al. (*54*), Stratilova et al. (*55*), Vora et al. (*56*), and Hyeon et al. (*57*). Polacsek and Pozsar (*52*) found that in all cases, carrot PME activities were much lower than those found in tomatoes. Markovic (*53*) extracted and partially purified carrot PME by ion-exchange chromatography on DEAE-Sephadex A 50 and subsequent chromatography on Sephadex G 75 and found three multiple forms of PME. However, no further studies on purified carrot PME have been reported yet.

The use of carrots as a base product for the formulation of mixed juices and baby foods is presently increasing. The conventional thermal processing schedule has a deteriorative effect on texture, flavor, and nutritional attributes. High-pressure processing, in contrast to high-temperature treatment, is specific so far as not or slightly affecting covalent bonds in the pressure range studied. Hence, high-pressure processing does not destroy natural flavors or colors (*58*). To obtain a stable product and eliminate detrimental effects caused by pressure stable enzymes, we recommended a thermal treatment combined with high-pressure processing. In this paper, PME from carrots was purified using a single-step affinity chromatography. Purified carrot PME was characterized in terms of biochemical properties and thermal and high-pressure stability.

MATERIALS AND METHODS

Materials. Carrots (Daucus carrota L.) were purchased from a local supermarket. Apple pectin [degree of esterification (DE) 75%] was obtained from Fluka Chemical Co. (Switzerland). CNBr-activated Sepharose 4B and commercial orange pectin methylesterase were purchased from Sigma (USA). Other chemicals were of analytical grade.

Kiwi PME inhibitor (PMEI) Extraction. The extraction of kiwi PMEI was performed according to the method of Giovane et al. (*59*, *60*). About 0.75 kg of ripe kiwi fruits was peeled and homogenized in water (1:1 w/v) at 4 °C. The suspension was centrifuged at 20 000*g*, 4 °C, for 20 min. The supernatant containing the glycoprotein inhibitor (PMEI) was separated from the pellet and adjusted to pH 6.0 using 1

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M NaOH. The supernatant was centrifuged once more to eliminate any small particles.

Carrot PME Extraction. The extraction of carrot PME was performed according to the method of Wicker (*61*). A sample of 1 kg of carrots was cut and homogenized, with the addition of 600 mL distilled water. The suspension was filtered using cheesecloth. The supernatant was discarded. The pellet was washed (twice) with 600 mL distilled water to remove other organic and color compounds and then mixed overnight in a 0.2 M Tris(hydroxymethyl)-aminomethan buffer (referred to as 'Tris' buffer) containing 1 M NaCl (pH 8.0) (1:1.3 w/v). After extraction, the suspension was filtered using cheesecloth, and the pellet was discarded. The salt extract was partly purified by ammonium sulfate precipitation at 30% saturation. After stirring for 30 min, the pellet after centrifugation at 18000*g* for 15 min was discarded. The supernatant was precipitated again by ammonium sulfate up to 80% saturation for 30 min. The precipitate containing PME was collected by centrifugation at 18000*g* for 15 min and dissolved in a 20 mM Tris buffer (pH 7.0), (volume of buffer used: 5 mL per 100 g of fresh material). This PME crude extract was further purified by affinity chromatography.

Carrot PME Purification by Affinity Chromatography. PME and PMEI matrixes were prepared according to the methods of Giovane et al. (*60*) and Denès et al. (*42*). To purify PMEI, we chemically immobilized commercial orange PME on the CNBr-Sepharose 4B resin. The resin (10 g) was swollen in 1 mM HCl and washed with 0.1 M Na_2CO_3 buffer (pH 8.3) containing 0.5 M NaCl. Commercial orange PME, dissolved in the same carbonate buffer, was added to the resin and agitated for 12 h at 4 °C. After this period, the PME-CNBr Sepharose 4B resin was mechanically mixed with 0.1 M Tris-HCl buffer (pH 8.0) for 2 h to deactivate the unoccupied sites. The resin was washed with water and stored in 0.2% sodium azide solution. This orange PME-CNBr Sepharose 4B resin was used for purification of kiwi PMEI.

The raw extract of kiwi containing PMEI was mixed with the PME-CNBr Sepharose 4B resin for 2 h at 4 °C. Afterward, the matrix was washed with distilled water and packed onto a chromatography column (XK16, Amersham Biosciences, Sweden) for affinity chromatography. The column was first washed with 75 mL of 2 mM KH_2PO_4 buffer (pH 6.0) containing 0.5 M NaCl, and the PMEI was recovered by eluting with 20 mM Na_2CO_3 buffer (pH 9.5) containing 1 M NaCl at a flow rate of 0.1 mL/min.

The fractions containing PMEI activity were pooled, diluted in 0.2 M $NaHCO_3$ buffer (pH 8.3) (1:1 v/v), and chemically immobilized on a second CNBr−Sepharose 4B resin (30 g) in the same way as that described for orange PME immobilization. The carrot PME crude extract was adjusted to pH 6.0 and agitated with PMEI-CNBr Sepharose 4B resin for 2 h at 4 °C. The gel was washed with water and packed onto another column (XK26, Amersham Biosciences, Sweden). The column was washed with 250 mL of 2 mM KH_2PO_4 buffer (pH 6.0) containing 0.5 M NaCl, and the carrot PME was recovered by eluting with 50 mM Na_2CO_3 buffer (pH 9.85) containing 1 M NaCl at a flow rate of 0.3 mL/min. Fractions containing PME activity were pooled and desalted using Centricon Plus-20 (PL-10) (Millipore). The concentrate obtained was dissolved in 20 mM Tris buffer (pH 7.0), quickly frozen using liquid nitrogen, and stored at −80 °C for all experiments.

PME Assay. PME activity was measured by continuous recording of the titration of carboxyl groups released from a pectin solution with 0.01 N NaOH using an automatic pH-stat (Metrohm, 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. The activity unit (*U*) of PME is defined as the amount of enzyme required releasing 1 μmol of carboxyl group per min, under the aforementioned assay conditions.

PMEI Assay. On the the basis of the stoichiometric reaction of PME and PMEI, samples of 50 μL of crude orange PME solution (7.5 mg/mL 20 mM Tris buffer, pH 7.0) were mixed with 100 μL of each fraction of PMEI purification. Mixtures were incubated for 15 min at room temperature. Afterward, PME activity of the mixtures was determined (as described in PME assay subsection). PMEI activity was calculated as the difference between PME activity of a blank sample (without PMEI) and residual PME activity of the mixtures.

Protein Determination. Protein concentration was determined using Sigma procedure No. TPRO-562 (for Kit No. BCA-1 and Product No. B-9643).

Gel Electrophoresis. A PhastSystem (Amersham Biosciences, Sweden) was used for both SDS−PAGE and IEF experiments. SDS−PAGE was performed using PhastGel homogeneous 20% and PhastGel Tris-tricine SDS buffer strips. Samples were boiled for 5 min at 100 °C in a buffer containing SDS (2.5%) and β-mercaptoethanol (5%). Proteins used as molar mass standards were phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-Lactalbumin (14.4 kDa). For IEF, PhastGel IEF media (polyacrylamide gels) with a pH range of 3−9 were used. Proteins used as pI standards were trypsinogen (pI-9.30), lentil lectin-basic band (pI-8.65), lentil lectin-middle band (pI-8.45), lentil lectin-acidic band (pI-8.15), myoglobin-basic band (pI-7.35), myoglobin-acidic band (pI-6.85), human carbonic anhydrase B (pI-6.55), bovine carbonic anhydrase B (pI-5.85), β-lactoglobulin A (pI-5.20), soybean trypsin inhibitor (pI-4.55), and amyloglucosidase (pI-3.50). Gel staining was performed with silver nitrate according to Heukeshoven and Dernick (*62*) using the equipment from Amersham Biosciences.

Thermal Inactivation of Purified Carrot PME. Thermal inactivation of purified carrot PME was investigated within a temperature range of 48−60 °C. Isothermal treatments were performed in a temperature controlled water bath using 200 μL-micro-pipets (Blaubrand, Germany) to enclose the enzyme solution. After treatments, micro-pipets were immediately cooled in ice water. Residual activities of PME were measured within 60 min of storage at 0 °C.

High-Pressure Inactivation of Purified Carrot PME. All pressure experiments were conducted in a multivessel high-pressure equipment (eight vessels of 8 mL) (Resato, Roden, The Netherlands), which allows pressurization up to 1000 MPa in combination with temperatures ranging from −20 to 100 °C. The pressure medium is a glycol−oil mixture (TR 15, Resato). Enzyme samples in flexible microtubes (0.3 mL, Elkay, Belgium) were enclosed in the pressure vessels, already equilibrated at a certain temperature. Pressure was built up slowly (100 MPa/min) to minimize temperature rise due to adiabatic heating. After pressure build up, an equilibration period of 2 min to allow temperature to evolve to its desired value was taken into account. At that moment, one pressure vessel was decompressed, and the activity of the corresponding enzyme sample was considered as the blank (A_0). The other seven vessels, each containing one enzyme sample, were then decompressed as a function of time. After pressure release, samples were immediately cooled in ice water, and the residual PME activity was measured within 60 min storage time at 0 °C. The pressure range studied varied from 600 to 700 MPa at 10 °C.

Kinetic Data Analysis. Inactivation of enzymes can often be described by a first-order kinetic model (*63*, *64*)

$$\ln\left(\frac{A_t}{A_0}\right) = -kt \tag{1}$$

where A_0 and A_t are the initial activity and the remaining activity at time *t*, respectively.

Equation 1 is valid under isothermal and isothermal−isobaric conditions, whereby the inactivation rate constant *k* can be determined from a linear regression analysis of $\ln(A_t/A_0)$ versus time.

A special case of a first-order model is a fractional conversion model. Fractional conversion *f* takes into account the nonzero activity after prolonged heating and/or pressurizing (=A_∞) and can be expressed mathematically as

$$\mathrm{f} = \frac{(A_0 - A_t)}{(A_0 - A_\infty)} \tag{2}$$

For most irreversible first-order reactions, A_∞ approaches zero, and eq 2 can be reduced to

$$f = \frac{(A_0 - A_t)}{A_0} \tag{3}$$

Table 1. Extraction and Purification of Carrot Pectin Methylesterase

	activity (U)	protein (mg)	specific activity (U mg^{-1})	recovery (%)	purification factor
crude extract	4536	2945	1.54	100	1
ammonium sulfate precipitation (80%)	3370	91.4	36.87	74.3	24
purified PME	2023[a]	5.97	338.83	44.6	220

[a] Pooled yield of purified carrot PME of two first purification runs from one stock of crude carrot extract.

A plot of the logarithm of $(1 - f)$ versus time yields a straight line with a rate constant expressed by the negative slope value (*65*):

$$\ln\left(\frac{A_t}{A_0}\right) = \ln(1 - f) = -kt \quad (4)$$

So, it is clear that eq 4 is identical to eq 1 when A_∞ approaches zero.

To account for the nonzero activity after prolonged heating and/or pressurizing, we should use fractional conversion in the following form:

$$\ln(1 - f) = \ln\left[\frac{(A_t - A_\infty)}{(A_0 - A_\infty)}\right] = -kt \quad (5)$$

Rearranging eq 5 yields eq 6. By plotting A_t (activity after different time intervals) versus inactivation time at constant pressure and/or temperature conditions, we can estimate the inactivation rate constant, k, and remaining activity, A_∞, using nonlinear regression analysis

$$A_t = A_\infty + (A_0 - A_\infty)\exp(-kt) \quad (6)$$

The accuracy of the fractional-conversion model was evaluated by calculation of corrected r^2 (eq 7) and standard errors of each estimated model parameter (*66*)

$$\text{corrected } r^2 = 1 - \frac{(m-1)\left(1 - \frac{\text{SSQ}_{\text{regression}}}{\text{SSQ}_{\text{total}}}\right)}{m - j} \quad (7)$$

where m = number of observations, j = number of model parameters, $\text{SSQ}_{\text{regression}}$ = sum of squares of "regression", and $\text{SSQ}_{\text{total}}$ = sum of squares of "uncorrected total".

It should be stressed that for the inactivation experiments at constant temperature and/or pressure, the heating, and/or pressurizing time should be long enough so that the remaining activity, A_∞, is no longer changing with respect to time (*67*, *68*).

Once 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:

$$\ln(k) = \ln(k_{\text{ref}}) + \left[\frac{E_a}{R}\left(\frac{1}{T_{\text{ref}}} - \frac{1}{T}\right)\right] \quad (8)$$

As a measure for the pressure dependence of the enzyme inactivation rate, the activation volume (V_a) of enzyme pressure inactivation can be estimated using the Eyring relationship:

$$\ln(k) = \ln(k_{\text{ref}}) - \left(\frac{V_a}{RT}(P - P_{\text{ref}})\right) \quad (9)$$

The activation energy and the activation volume can be estimated by linear regression analysis of the logarithm of the rate constant versus the inverse of absolute temperature or pressure, respectively.

RESULTS AND DISCUSSION

Extraction and Purification of Carrot PME. After centrifugation of homogenized carrot suspension, no PME activity was detected in the supernatants. Therefore, it can be stated that carrot PME is bound by electrostatic interaction to the cell walls and that Tris buffer with high ionic strength [0.2 M Tris containing 1 M NaCl (pH 8.0)] is suited for the extraction of the enzyme. The precipitation of PME from the carrot crude extract using ammonium sulfate at 30% and 80% saturation allows to (i) remove inert proteins from the concentrate, (ii) separate proteins from crude extract solution before binding to the gel, which otherwise pollutes the matrix, and (iii) easily set up the volumetric ratio of protein solution:gel (2:1) as an optimal binding condition. **Table 1** shows the increase in purification factor (from 1 to 24) after the ammonium sulfate precipitation (80%) step corresponding with 25% loss of PME activity.

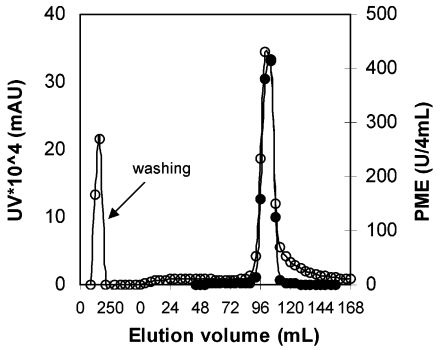

Figure 1. Elution profile of carrot PME on PMEI-CNBr Sepharose 4B column. Washing solution was 2 mM KH_2PO_4 containing 0.5 M NaCl pH 6.0. Elution buffer was 50 mM Na_2CO_3 containing 1 M NaCl pH 9.85. UV absorbance (measured at λ = 280 nm) (○) and PME activity (●).

In **Figure 1**, the elution profile of carrot PME shows that inert proteins were washed out using 2 mM KH_2PO_4 buffer (pH 6.0) containing 0.5 M NaCl. Purified carrot PME was eluted with a high ionic strength and high pH buffer, and a single peak of proteins and PME activity was obtained. The maximum binding capacity of the column (30 g of gel) was around 1000−1100 PME units. Filtrate containing PME activity collected after binding PME crude extract on the gel could be reused for sequential purification runs. Each replicate purification of the enzyme from the crude carrot extract showed a single peak. Purified carrot PME had a maximum activity of 338.8 U mg^{-1} protein, corresponding to an at least 220-fold enrichment and an overall yield (of two purification runs) of at least 44.6%, based on the total enzymatic activity in the crude extract (**Table 1**).

Electrophoresis. Carrot PME showed three bands on SDS−PAGE (**Figure 2**). A comparison using ImageMaster 1D software (Amersham Biosciences, Sweden) based on standard proteins indicated two major bands of similar intensity corresponding to apparent molar masses (MM) of 34.5 and 44 kDa and a minor band of 36.3 kDa. The fourth thin band with MM of 76.7 kDa appearing on top of the gel (**Figure 2**) is due to a contaminant in the sample buffer. Markovic (*53*) reported three multiple forms of carrot PME that thin-layer chromatography on Sephadex G 150 superfine showed a single spot of enzyme activity of MM 27 kDa.

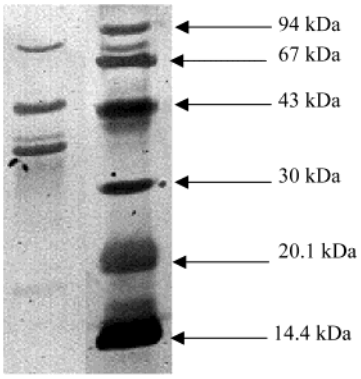

Figure 2. SDS–PAGE of carrot PME: (A) carrot PME after affinity chromatography, (B) MM standards.

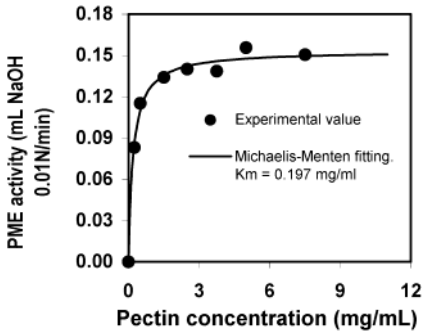

Figure 3. Activity of carrot PME as a function of substrate concentration. Assay conditions: apple pectin (DE 75%), pH 7.0, 35 °C, 0.117 M NaCl.

On IEF gel, no protein band was found between pH 3.0 and 9.0. The isoelectric point of carrot PME is higher than 9.0, as reported for PMEs of tomato [9.3, (*69*)], orange [10.05, 10.2 and >11 (*18*), 9.18 (*37*)], grapefruit [>10 (*22*)], banana [9.3−9.4 (*51*)], papaya [>9 (*70*)], and apple [>9 (*42*)].

Purified Carrot PME Kinetic Parameters. *Effect of Substrate Concentration.* The kinetic parameter, K_m,of carrot PME was determined by measuring enzyme activity at different pectin concentrations (**Figure 3**). Activity assays were performed using apple pectin solution (DE 75%) up to 7.5 mg/mL containing 0.117 M NaCl. By fitting the Michaelis−Menten model on the experimental data, we found a K_m of 0.197 mg/mL pectin solution for carrot PME. Denès et al. (*42*) obtained a K_m of 0.098 mg/mL pectin solution for purified apple PME (assay condition: apple pectin (DE 75%), pH 7.0, 35 °C, 0.1 M NaCl).

Effect of pH and Temperature. Optimal pH and temperature for carrot PME activity were determined in a temperature range of 40−60 °C and a pH range of 6.5−8.5. Activity assays were performed using apple pectin solution (DE 75%) at 3.5 mg/mL containing 0.117 M NaCl, taking into account the autohydrolysis of pectin at elevated temperature and pH levels. Statgraphics Plus (version 5) software was used for the multiple regression analysis involved in the modeling (*14*). PME activity response surface and contour plots were made using SAS software to depict the effects of pH and temperature on carrot PME activity (**Figure 4**).

In **Figure 4** purified carrot PME activity increased to an optimum at pH 7.3−7.4 and a temperature of 48.5 °C. Denès et al. (*42*) found about the same optimal pH (>7.5) for purified apple PME (cv Golden Delicious). Lee and Wiley (*45*) reported an optimal temperature of 55 °C and pH between 6.5 and 7.5 for apple PME, whereas Puri et al. (*19*) reported an optimal pH

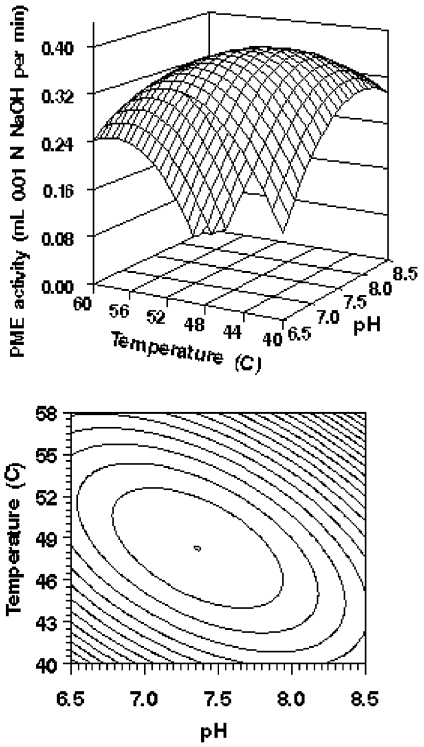

Figure 4. Carrot PME activity response surface and contour plots as functions of pH and temperature. Assay conditions: apple pectin (DE 75%) solution 3.5 mg/mL, 0.117 M NaCl.

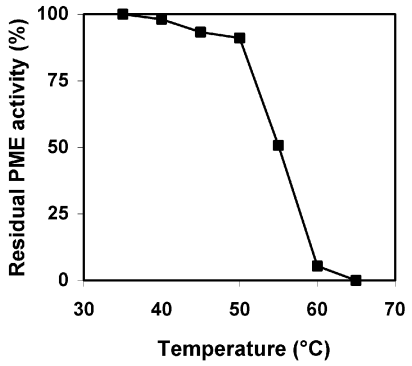

Figure 5. Thermal stability of purified carrot PME. The residual activity was measured after 10 min treatment at different temperatures.

and temperature of 7.5 and 55 °C, respectively, for partially purified potato PME (cv. Russet Burbank). For PMEs of other sources, an optimal pH of 8.0 was found for PMEs of papaya (*20*) and of peach (*71*).

Thermal Stability. Samples of purified carrot PME dissolved in 20 mM Tris buffer (pH 7.0) were heated for 10 min at temperatures varying from 35 to 65 °C. In **Figure 5**, relative residual PME activity after heating is plotted as a function of inactivation temperature. Within the temperature range of inactivation, purified carrot PME was gradually inactivated. Above 50 °C, a significant loss of carrot PME activity was observed. At 55 °C, 50% of PME activity was lost after 10 min of treatment, whereas at 60 °C about 95% of PME activity was lost under the same conditions.

Thermal Inactivation Kinetics of Purified Carrot PME. Isothermal inactivation, at atmospheric pressure, of purified carrot PME [dissolved in 20 mM Tris buffer (pH 7.0)] could be accurately described by a fractional-conversion model in a temperature range of 48−60 °C (**Figure 6**). The thermostable carrot PME fraction attributed to about 8% of the total activity.

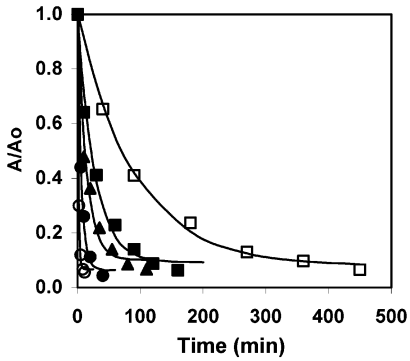

Figure 6. Thermal inactivation of purified carrot PME dissolved in 20 mM Tris buffer (pH 7.0) at 48 (□), 51 (■), 54 (▲), 57 (●), and 60 °C (○).

Table 2. Kinetic Parameter Estimates of a Fractional-Conversion Model for Isothermal Inactivation of Purified Carrot PME at Atmospheric Pressure

T (°C)	k (min^{-1})	corrected r^2	A_∞ (%)
48	0.0110 ± 0.0006[a]	0.999	7.8 ± 1.4
51	0.0375 ± 0.0046	0.993	9.2 ± 2.8
54	0.0701 ± 0.0083	0.992	10.1 ± 2.7
57	0.1694 ± 0.0129	0.998	6.3 ± 2.1
60	0.6814 ± 0.0361	0.999	6.7 ± 1.0
	E_a = 289.2 ± 22.0 kJ/mol	R^2_{Ea} = 0.983[b]	

[a] Standard error of regression. [b] Regression coefficient of the Arrhenius equation.

Further separation of carrot PME isozymes and thermal stability studies of individual isozymes might be useful to gain insight in the observed fractional-conversion behavior during thermal processing. Inactivation rate constants, estimated using nonlinear regression analysis of eq 6, are reported (**Table 2**). As expected, the inactivation rate constants increase with increasing temperatures. Purified carrot PME is less stable toward thermal treatment compared to PMEs from other sources. The inactivation rate constant at 60 °C of purified carrot PME is 0.6814 min^{-1}. Van den Broeck et al. (*67*, *68*) obtained inactivation rate constants k_{60} °C between 0.103 and 0.165 min^{-1} for commercial orange PME and between 0.055 and 0.112 min^{-1} for orange crude extract PME, both thermally treated in water. The same authors (*68*) found inactivation rate constants k_{60} °C between 0.035 and 0.174 min^{-1} for orange crude extract PME, when thermally treated in citric acid buffers at a pH range of 3.2−4.2.

The temperature dependence of inactivation rate constants in the temperature range studied could be adequately described by the Arrhenius equation (**Figure 7**), yielding an activation energy of 289.2 kJ/mol. This value is somewhat lower than those obtained by Van den Broeck et al. (*67*) (301.4−350.5 kJ/mol) for commercial orange PME. Massaguer et al. (*72*) also reported activation energy levels of 61.7 kcal/mol (257.9 kJ/mol) and 72.7 kcal/mol (303.9 kJ/mol) for the thermal inactivation of heat-labile and heat-stable PME from acidified papaya pulp.

High-Pressure Inactivation Kinetics of Purified Carrot PME. Adiabatic heating during pressurization is a potential source of thermal enzyme destruction. To avoid such a problem, we conducted pressure experiments at rather low temperature (i.e., 10 °C). Like high-pressure inactivation of orange PME (*67*, *68*), the high-pressure inactivation at 10 °C of purified carrot PME could be adequately described by a fractional-conversion model in the pressure range of 600−700 MPa (**Figure 8**), indicating the presence of a first-order inactivating pressure-sensitive carrot PME fraction and the occurrence of a pressure-stable PME fraction.

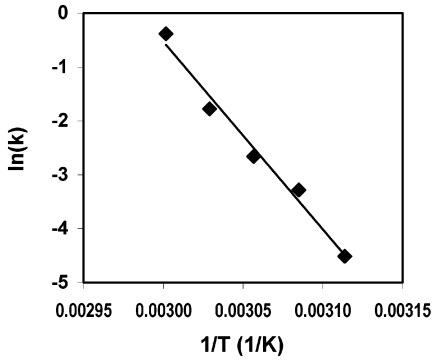

Figure 7. Temperature dependence of inactivation rate constant for thermal inactivation of purified carrot PME.

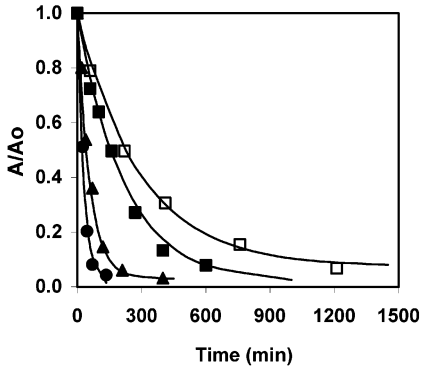

Figure 8. High-pressure inactivation at 10 °C of purified carrot PME dissolved in 20 mM Tris buffer (pH 7.0), modeled using a fractional-conversion model: 600 (□), 625 (■), 675 (▲), and 700 MPa (●).

Table 3. Kinetic Parameter Estimates of a Fractional-Conversion Model for Isothermal–Isobaric Inactivation of Purified Carrot PME at 10 °C

P (MPa)	k (min^{-1})	corrected r^2	A_∞ (%)
600	0.0034 ± 0.0002[a]	0.998	7.4 ± 2.4
625	0.0045 ± 0.0004	0.998	1.5 ± 3.6
675	0.0175 ± 0.0006	0.999	2.7 ± 1.1
700	0.0314 ± 0.0045	0.993	0.03 ± 4.8
	V_a = − 54.7 ± 4.6 cm^3/mol	R^2_{Va} = 0.986[b]	

[a] Standard error of regression. [b] Regression coefficient of the Eyring equation.

The inactivation rate constants of the pressure-sensitive carrot PME fraction are to increase with increasing pressure levels applied (**Table 3**). The pressure-stable PME fraction attributed more or less 3% of the total activity. Goodner et al. (*73*) found that high-pressure (>600 MPa) caused instantaneous inactivation of the heat-labile form of PME in orange and grapefruit juices but did not inactivate the heat-stable form of PME.

The pressure dependence of the inactivation rate constants of the pressure-labile PME fraction within the pressure range investigated could be adequately modeled by the Eyring relationship, yielding an activation volume of −54.7 cm^3/mol (**Figure 9**). The absolute value of this measure is much higher than those obtained by Van den Broeck et al. (*67*) for orange PME (−24.55 to −29.29 cm^3/mol).

In conclusion, carrot PME can be successfully purified by a single-affinity chromatography step using a PME-inhibitor isolated from kiwi fruit. Isothermal and isobaric−isothermal inactivation of purified carrot PME follows fractional-conversion

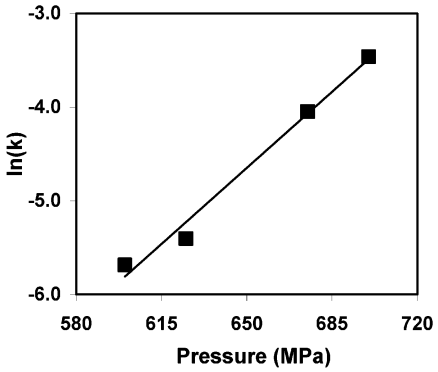

Figure 9. Pressure dependence of inactivation rate constant for high-pressure inactivation of purified carrot PME.

models. Carrot PME is more sensitive to heat treatments as compared to PME from oranges.

NOMENCLATURE

List of Abbreviations

PME	pectin methylesterase
PMEI	pectin methylesterase inhibitor
Tris	tris(hydroxymethyl)-aminomethan
SDS−PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
IEF	isoelectric focusing
MM	molar mass

List of Symbols

A_t	enzyme activity at time t (mL 0.01 N NaOH/min)
A_0	initial enzyme activity (mL 0.01 N NaOH/min)
A_∞	enzyme activity when the reaction time is very long (mL 0.01 N NaOH/min)
E_a	activation energy (kJ/mol)
f	fractional conversion
k	first-order inactivation rate constant (min^{-1})
k_{ref}	first-order inactivation rate constant at a reference temperature or pressure (min^{-1})
P	pressure (MPa)
P_{ref}	reference pressure (MPa)
R	universal gas constant (8.3143 J/mol K)
t	time (min)
T	temperature (K)
T_{ref}	reference temperature (K)
U	unit of pectin methylesterase
V_a	activation volume (cm^3/mol)

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