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Purification and Thermal and High-Pressure Inactivation of Pectinmethylesterase Isoenzymes from Tomatoes (*Lycopersicon esculentum*): A Novel Pressure Labile Isoenzyme

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Tomato pectinmethylesterase (PME) was successfully purified by a two-step method consisting of affinity chromatography followed by cation exchange chromatography. According to this procedure, four different isoenzymes were identified representing molar masses around 34.5–35.0 kDa. Thermal and high-pressure inactivation kinetics of the two major isoenzymes of tomato PME were studied. A striking difference between their process stability was found. The thermostable isoenzyme was completely inactivated after 5.0 min at 70 °C, whereas for the thermolabile isoenzyme, temperatures at around 60 °C were sufficient for complete inactivation. The thermostable isoenzyme was also found to be pressure stable since no inactivation was observed after 5.0 min of treatment at 800 MPa and 20 or 40 °C. The thermolabile isoenzyme appeared to be pressure labile since it could be completely inactivated after 5.0 min of treatment at 700 MPa and 20 °C or 650 MPa and 40 °C. Inactivation kinetics at pH 6.0 could be accurately described by a first-order model.

KEYWORDS: ***Lycopersicon esculentum*; tomatoes; pectinmethylesterase; isoenzymes; thermal stability; high-pressure stability; inactivation kinetics**

INTRODUCTION

Tomatoes (*Lycopersicon esculentum*), the second-most consumed vegetable around the world, can be consumed either as fresh or as processed products such as juices, pastes, purees, ketchup, sauces, and soups (*1*). Traditional thermal treatment remains the most widely used method for food processing. This treatment allows for the obtaining of safe foods; however, it may cause undesirable losses of sensory and nutritional quality. Nowadays, due to a growing consumer demand for minimally processed foods, high-pressure processing is gaining interest as an alternative or complement to thermal processing. This new technology allows the inactivation of microorganims (*2*, *3*) and may induce either activation or inactivation of quality related enzymes (*4*), while better retaining quality attributes of fresh foods such as flavor and nutrients (*5*–*7*).

In addition to microbial safety and health-related aspects, important quality properties of tomato products are color, flavor, and consistency. Changes in consistency of tomato products during industrial processing are closely related to modifications in the structure of pectin, a major plant cell wall polysaccharide, as a result of transformations facilitated by a series of pectolytic enzymes and chemical breakdown of the cell wall material (*6*). One of these pectolytic enzymes involved is pectinmethylesterase (PME; EC 3.1.1.11). PME is a cell-wall-bound enzyme that catalyzes the de-esterification of pectin, yielding acidic pectin with a lower degree of esterification and methanol. Several isoenzymes of tomato PME that differ in molar mass and biochemical properties have been reported (*8*–*11*). The primary structure of the major isoenzyme (*12*) and its 3D structure (*13*) have been elucidated.

The variability in processing stability of PME isoenzymes is important in many food industries. Hence, a better knowledge of the thermal and high-pressure stability of PME isoenzymes and kinetic data is indispensable in order to implement and optimize these processes. In this sense, most of the available studies in the literature about tomato PME have focused only on the main isoenzyme (*14*–*16*). However, there is scarce information regarding the thermostability of different isoenzymes of tomato PME (*17*), and there are no studies available comparing their high-pressure stability. A pressure-labile isoenzyme of tomato PME has so far not been reported. Therefore, the main objectives of this work were to purify different PME

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isoenzymes from tomato fruit and to study the thermal and high-presssure inactivation kinetics of the two major isoenzymes.

MATERIALS AND METHODS

Raw Material. PME was extracted from whole ripe tomatoes (*Lycopersicon esculentum*, variety Perfectpeel). Tomato fruits were purchased from a local supermarket, cut, frozen in liquid nitrogen, and stored at –40 °C until use.

Tomato PME Extraction. The extraction of tomato PME was performed according to the method of Fachin et al. (*14*) with some minor modifications. A sample of 1 kg of thawed tomatoes was homogenized in a blender, without the addition of water, and centrifuged at 10000*g* for 30 min. The supernatant was discarded, while the pellet was mixed end-over-end for 2 h with 0.2 M Tris-HCl buffer containing 1.0 M NaCl (pH 8.0; 1:2.5 w/v) for the extraction of cell-wall-bound PME. Afterwards, the salt extract was collected by centrifugation at 10000*g* for 60 min and subjected to ammonium sulfate precipitation. The fraction precipitating between 30% and 80% ammonium sulfate saturation was collected by centrifugation at 18000*g* for 15 min and dissolved in 20 mM Tris-HCl buffer (pH 7.5; 5 mL buffer/100 g of fresh material). This crude extract, containing PME, was stored frozen until further purification by affinity chromatography. All the steps of this procedure were performed at 4 °C.

Tomato PME Purification. The purification of PME from tomato crude extract was carried out using a two-step method consisting of affinity chromatography followed by cation exchange chromatography.

Affinity Chromatography. Tomato PME was purified by affinity chromatography (AKTA Prime, GE Healthcare, Uppsala, Sweden) using a PME inhibitor (PMEI) that was extracted and purified from kiwi fruit according to the method described by Guiavarc'h et al. (*18*) and chemically immobilized on a N-hydroxysuccinimide (NHS)-activated Sepharose 4 Fast Flow matrix (GE Healthcare, Uppsala, Sweden). The resin (25 mL) was swollen in 1 mM HCl and washed with a 0.1 M Na_2CO_3 buffer (pH 8.3) containing 0.5 M NaCl. The purified kiwi PMEI, dissolved in the same carbonate buffer (0.1 M, pH 8.3), was added to the swollen resin and agitated end-over-end overnight at 4 °C. Afterwards, the kiwi PMEI–NHS-activated Sepharose 4 Fast Flow matrix was mixed with a blocking buffer (0.1 M Tris-HCl, pH 8.0) for 2 h to deactivate the unoccupied sites. After washing with a high-pH buffer (0.1 M Tris-HCl, pH 8.5, + 0.5 M NaCl), a low-pH buffer (0.1 M sodium acetate, pH 4.5, + 0.5 M NaCl), and deionized water, the gel was ready for purification of the tomato PME. The crude extract containing tomato PME was mixed end-over-end with the kiwi PMEI–NHS-activated Sepharose 4 Fast Flow matrix for 6 h at 4 °C. Afterwards, the PME–PMEI–NHS Sepharose gel was washed with deionized water and packed onto a chromatography column (XK16, GE Healthcare, Uppsala, Sweden). The column was first washed with 75 mL of a 2.0 mM KH_2PO_4 buffer (pH 6.0) containing 0.5 M NaCl. The PME was recovered by eluting with a 50 mM Na_2CO_3 buffer (pH 9.85) containing 1.0 M NaCl at a flow rate of 0.1 mL/min. After elution, the fractions possessing PME activity were pooled and desalted using Vivaspin 20 centrifugal filters (Vivascience, Hannover, Germany). The tomato PME concentrate obtained was dissolved in a 50 mM sodium acetate buffer (pH 5.5) and used for further purification by cation exchange chromatography.

Cation Exchange Chromatography. After affinity chromatography, 5 mL of the extract containing tomato PME was filtered using a 0.45 μm syringe-driven filter (Millipore, Billerica, MA) and applied to a cation exchange column (Hi Prep 16/10 SP/XL, GE Healthcare, Uppsala, Sweden). The column was previously equilibrated with a 50 mM sodium acetate buffer (pH 5.5). The elution of bound proteins was performed with a linear salt gradient to 1.0 M NaCl in a sodium acetate buffer (pH 5.5), at a flow rate of 0.5 mL/min for 10 column volumes. Active fractions were pooled, desalted, and concentrated.

PME Activity Assay. PME activity was determined by measuring the release of acid per time unit at a constant pH and temperature (pH 7.0 and 22 °C). The reaction mixture consisted of 250 μL of enzyme sample and 30 mL of a 3.5 g/L apple pectin solution (DM 70–75%, Fluka) containing 0.117 M NaCl. During pectin hydrolysis, the pH was maintained constant by the addition of 0.01 M NaOH using an automatic pH-stat titrator (Titrino 718, Metrohm, Switzerland). The PME activity is proportional to the rate of base consumption. One unit (U) of PME activity is defined as the amount of enzyme capable of catalyzing the hydrolysis of 1 μmol of methyl-ester bonds per minute at a constant pH and temperature.

Protein Content. The protein content was determined using the bicinchoninic acid (BCA) kit according to Sigma procedure number TPRO-562 (Sigma, Darmstadt, Germany). This method is based on the reduction of Cu^{2+} to Cu^{+} by proteins in an alkaline environment. Then, BCA reacts with Cu^{+} to form a complex with an intense purple color at 562 nm. The protein concentration (mg/mL) was calculated by comparison with a standard curve using bovine serum albumin (Sigma, Darmstadt, Germany).

SDS-PAGE and Isoelectric Focusing. A PhastSystem (GE Healthcare, Uppsala, Sweden) was used for SDS-PAGE and isoelectric focusing (IEF) experiments. SDS-PAGE under denaturing conditions was performed using PhastGel homogeneous 20% and PhastGel Tris-tricine SDS buffer strips, whereas IEF was carried out using PhastGel IEF for a pH range of 3.0–9.0 (GE Healthcare, Uppsala, Sweden).

For both experiments, gel staining was performed with silver staining according to the procedure of Heukeshoven and Dernick (*19*). The molar mass and the isoelectric pH of the samples were derived by comparing the migration distance with a calibration curve of marker proteins using IMAGEMASTER 1D software (GE Healthcare, Uppsala, Sweden).

MALDI-TOF Mass Spectrometry. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to determine the molar mass of PME isoenzymes. MALDI-TOF MS was performed on a Reflex IV (Bruker Daltonic GmbH), equipped with a N_2 laser and pulsed ion-extraction accessory. In each case, 1 μL of the sample was transferred to a steel target, mixed with 1 μL of a saturated solution of α-cyano-4-hydroxycinnamic acid in acetone, and air-dried. The instrument was calibrated using a standard protein mixture (Bruker Daltonic GmbH). Positive ion spectra were recorded in the linear mode.

Thermal Treatment. Isothermal treatments of two major PME isoenzymes purified from tomato fruit were performed in a temperature-controlled water bath. Experiments were carried out in a temperature range from 20 to 80 °C for the screening of thermal stability (treatment time of 5.0 min) and from 51 to 69 °C for the isothermal inactivation studies (as a function of time). Samples were dissolved in a 0.10 M citrate buffer at pH 4.0 or pH 6.0, or in a 0.10 M phosphate buffer at pH 8.0. In order to ensure isothermal heating, the enzyme solution was enclosed in capillary tubes (1.15 mm × 150 mm, Hirschmann Laborgerate, Germany) by means of a vacuum pump. After treatment, samples were immediately cooled in ice water to stop the effect of heat. The residual PME activity was measured within 60 min of storage at 0 °C. No reactivation of the enzyme was observed during this period.

High-Pressure Treatment. Isothermal–isobaric treatments of two major PME isoenzymes purified from tomato fruit were carried out using laboratory-scale multivessel high-pressure equipment (HPIU-10000, Resato, Roden, The Netherlands). This equipment consists of eight individual vessels and allows pressurization up to 1000 MPa in combination with temperatures ranging from –20 to +100 °C. The pressure medium was a glycol–oil mixture (TR 15, Resato, Roden, The Netherlands). The temperature was controlled by a thermostated mantel which surrounds each vessel and is connected to a cryostat. Flexible microtubes (Biozym, Landgraaf, The Netherlands) were filled with 0.3 mL of PME solution and enclosed in the pressure vessels, already equilibrated at a preset temperature. Pressure was built up slowly at 100 MPa/min to minimize adiabatic heating. After the desired pressure was reached, an equilibration period of 4.0 min to ensure isothermal conditions was taken into account. At this moment, the pressure in one vessel was released and the enzyme activity of this sample was considered as the blank (A_0). After preset holding times, the other vessels were depressurized, samples were immediately cooled in ice water, and the residual PME activity was measured. Samples, dissolved in a 0.10 M citrate buffer at pH 6.0, were subjected to pressures from 100 to 800 MPa (5 min for the screening of high-presssure stability or preset holding times for the isothermal–isobaric inactivation studies), at 20.0 or 40.0 °C.

Table 1. Nomenclature of Tomato PME Samples during the Purification Process

symbol	meaning
AC_1	1st peak eluted after affinity chromatography
AC_2	2nd peak eluted after affinity chromatography
AC_{1+2}	1st peak + 2nd peak eluted after affinity chromatography
CE_1	1st peak eluted after cation exchange chromatography of AC_{1+2}
CE_2	2nd peak eluted after cation exchange chromatography of AC_{1+2}
$CE_{1.1}$	1st peak eluted after cation exchange chromatography of AC_1
$CE_{2.1}$	1st peak eluted after cation exchange chromatography of AC_2
$CE_{2.2}$	2nd peak eluted after cation exchange chromatography of AC_2
$CE_{2.3}$	3rd peak eluted after cation exchange chromatography of AC_2
$CE_{2.4}$	4th peak eluted after cation exchange chromatography of AC_2

Data Analysis. Enzyme inactivation under isothermal or isobaric–isothermal conditions can often be described by a first-order kinetic model (*20*):

$$\ln A = \ln A_0 - kt \quad (1)$$

where A is the enzyme activity at time t, A_0 is the enzyme activity at time zero, k is the inactivation rate constant, and t is the treatment time.

In the area of food processing, it is common to characterize first-order reactions by means of D and z values (thermal death time concept). The decimal reduction time (D value) is defined as the time, at a given temperature and pressure, needed for a 90% reduction of the initial activity. For first order reactions, D values and rate constants are directly related:

$$D = \frac{\ln 10}{k} \quad (2)$$

The decimal reduction time at a given inactivation temperature and pressure can be estimated from the slope of a linear regression analysis of $\log(A/A_0)$ versus treatment time:

$$\log\left(\frac{A}{A_0}\right) = -\frac{t}{D} \quad (3)$$

The temperature dependence of the D value is given by the Z_T value, whereas the pressure dependence of the D value is given by the Z_P value. The Z_T value (°C), at a certain pressure, equals the temperature increase necessary to obtain a 10-fold decrease of the decimal reduction time:

$$D = D_{ref} \times 10^{(T_{ref}-T)/Z_T} \quad (4)$$

The Z_p value (MPa), at a certain temperature, is defined as the pressure increase necessary to obtain a 10-fold decrease of the decimal reduction time:

$$D = D_{ref} \times 10^{(P_{ref}-P)/Z_P} \quad (5)$$

In this work, the Z_T and Z_P values were estimated by global nonlinear regression analysis, considering the inactivation data obtained at different inactivation temperatures or pressures simultaneously and incorporating the temperature or pressure coefficient model in the inactivation rate eq 3.

RESULTS AND DISCUSSION

Purification and Characterization of Tomato PME. Cell-wall-bound tomato PME was extracted from ripe tomatoes using a high-ionic-strength buffer, followed by ammonium sulfate precipitation of the salt extract to remove contaminating substances. Purification of the tomato PME was performed using a two-step method including, first, affinity chromatography and, then, cation exchange chromatography. **Table 1** shows the nomenclature used during the purification process. A summary of the purification of tomato PME is reported in **Table 2**. After affinity chromatography, the elution profile of tomato PME presented two overlapping peaks of PME activity, AC_1 (peak eluted first, fraction 14) and AC_2 (peak eluted second, fraction 17) (**Figure 1A**). When the fractions from AC_1 and AC_2 were analyzed on SDS-PAGE, only one band was observed for each sample, corresponding in both cases to a molar mass of 35.0 kDa (data not shown). The isoelectric pH for purified tomato PME (pool of fractions of PME activity, fractions 14 and 17, AC_{1+2}) was higher than 9 (data not shown). These results are in agreement with those reported by other authors (*10*, *14*, *21*). Baseline separation of AC_1 and AC_2 did not occur after the affinity chromatography step; thus, an additional step was necessary.

Table 2. Summary of the Purification of Tomato PME

sample	activity (U/mL)	protein content (mg/mL)	specific activity (U/mg)	purification factor[a]
crude extract	847	12.74	66	1.0
AC_{1+2}	138	0.19	744	11.2
CE_1	189	0.23	847	12.7
CE_2	58	0.07	834	12.6

[a] Calculated as the ratio of specific activity.

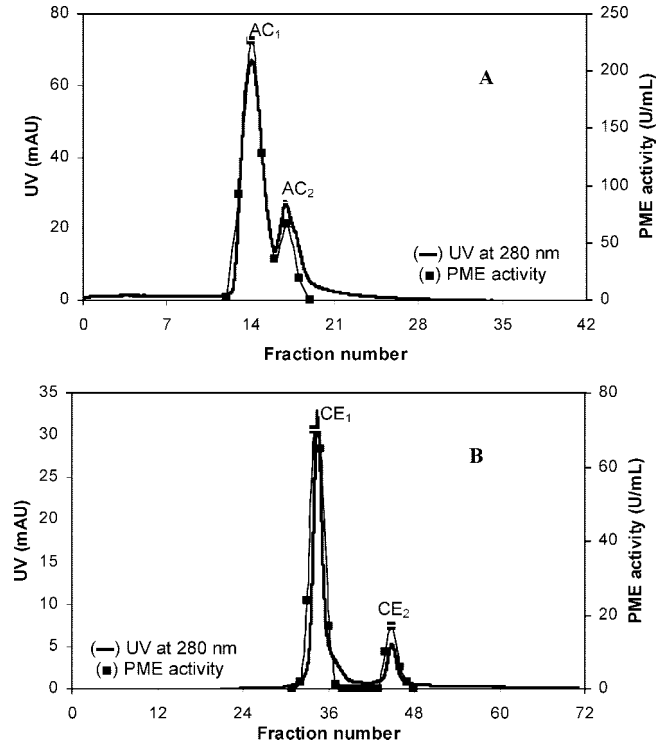

Figure 1. (A) Affinity chromatography elution profile of tomato PME. **(B)** Cation exchange chromatography elution profile of tomato PME from AC_{1+2}.

Cation exchange chromatography of the pooled fractions after affinity chromatography (AC_{1+2}) was performed. Fractions of PME activity were collected in two pools, corresponding to the peak eluted first (CE_1) and the peak eluted second (CE_2) (**Figure 1B**). CE_1 and CE_2 represented around 85% and 15% of the PME activity, respectively. **Figure 2** shows a single protein band of 34.5 kDa on SDS-PAGE for both CE_1 and CE_2. To identify other possible isoenzymes that could exist together with the two main ones but in minor proportions, AC_1 and AC_2 were injected separately into the cation exchange column. After the cation exchange chromatography of AC_1, a single sharp protein peak was observed ($CE_{1.1}$; data not shown). On the other hand, as

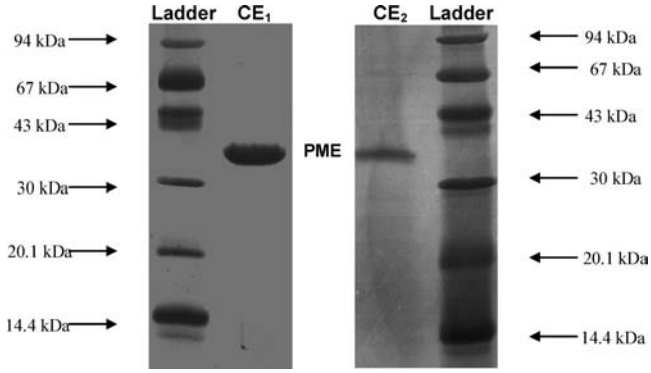

Figure 2. SDS-PAGE of tomato PME after cation exchange chromatography.

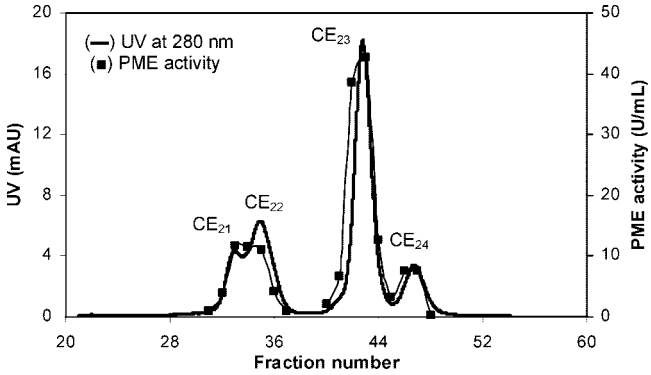

Figure 3. Cation exchange chromatography elution profile of tomato PME from AC_2.

shown in **Figure 3**, after the cation exchange chromatography of AC_2, four different peaks appeared ($CE_{2.1}$, $CE_{2.2}$, $CE_{2.3}$, and $CE_{2.4}$), which are assumed to have different charge densities. Under the same chromatographic conditions, $CE_{2.1}$ presented

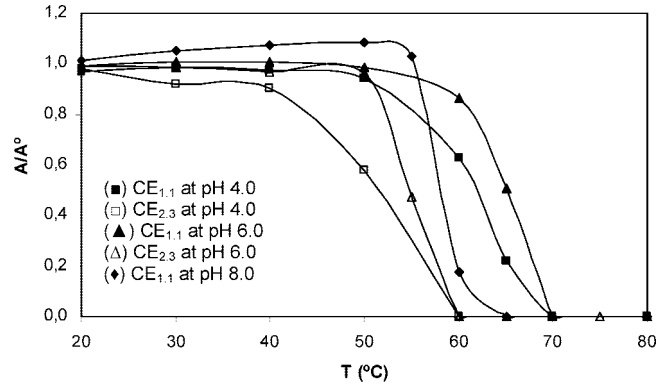

Figure 5. Residual activity of purified tomato PME as a function of pH after 5.0 min of treatment at preset temperatures.

the same elution time as $CE_{1.1}$ and CE_1, whereas $CE_{2.3}$ had the same elution time as CE_2, thus corresponding to the same isoenzyme in each case. MALDI-TOF MS for $CE_{2.1}$, $CE_{2.2}$, $CE_{2.3}$, and $CE_{2.4}$ was carried out (**Figure 4**), showing molar masses of 34.5, 34.4, 34.5, and 35.0 kDa, respectively. These values were in accordance with those obtained by SDS-PAGE for CE_1 and CE_2. Therefore, on the basis of the purification procedure used, four different isoenzymes of tomato PME were identified, two of them being the most abundant, $CE_{1.1}$ and $CE_{2.3}$. This number of isoenzymes is in agreement with that reported by Warrilow et al. (*11*) in tomato fruit. However, a multiple number of PME isoenzymes has been reported in the literature depending on the product, variety, degree of maturity, method of extraction, and purification procedure (*9*, *10*, *22–25*).

Thermal Inactivation of Tomato PME. The thermostability of two major isoenzymes of tomato PME, $CE_{1.1}$ and $CE_{2.3}$, was tested at different pHs (pHs 4.0, 6.0, and 8.0). As shown in **Figure 5**, the pH of the medium had a pronounced influence. $CE_{1.1}$ presented the highest thermostability at pH 6.0 (inactiva-

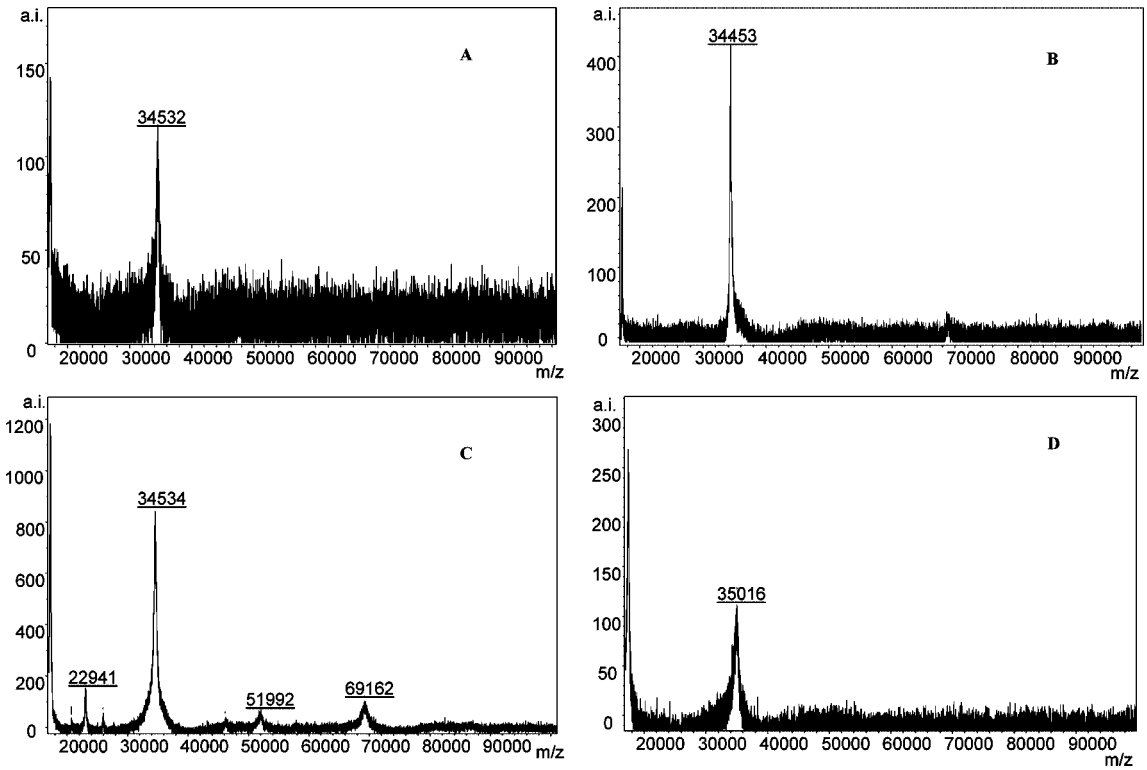

Figure 4. MALDI-TOF mass spectra of purified tomato PME. **(A)** $CE_{2.1}$, **(B)** $CE_{2.2}$, **(C)** $CE_{2.3}$, **(D)** $CE_{2.4}$ (*m*/*z* is mass-to-charge ratio, a.i. is absolute intensity).

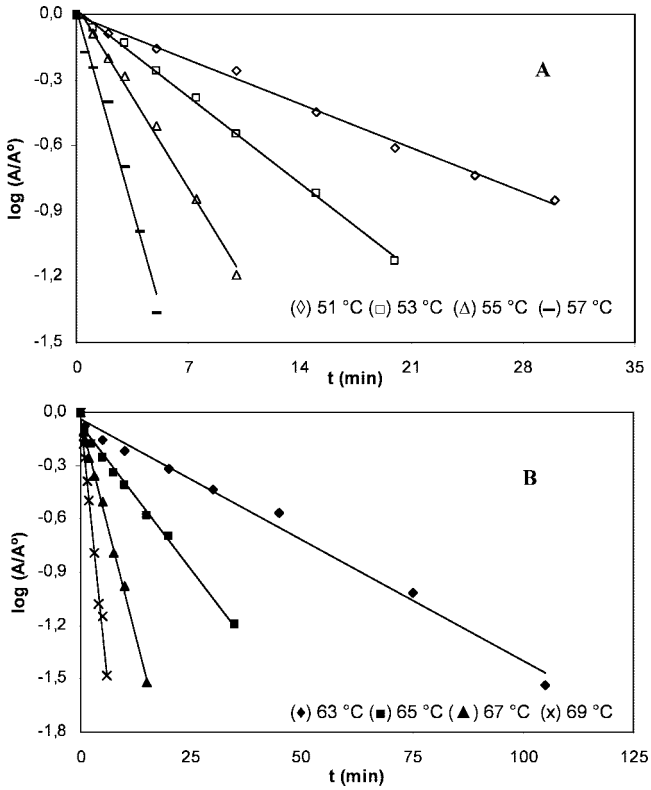

Figure 6. Thermal inactivation of purified tomato PME, **(A)** $CE_{2.3}$ and **(B)** $CE_{1.1}$, in 0.10 M citric acid buffer at pH 6.0.

tion between 60 and 70 °C), whereas the lowest stability was observed at pH 8.0 (inactivation between 55 and 65 °C). At pH 4.0, its thermostability was slightly lower than at pH 6.0 but higher than at pH 8.0. A similar trend was observed for $CE_{2.3}$ at pHs 4.0 and 6.0. Crelier et al. (*26*) reported that tomato PME was less sensitive to both heat and pressure when the pH was raised from 4.2 to 7.0. When the thermostability of both isoenzymes was compared at pH 6.0, $CE_{2.3}$ was completely inactivated after 5.0 min at 60 °C while, for $CE_{1.1}$, temperatures around 70 °C were necessary for complete inactivation. Several authors have reported similar results for the major isoenzyme of tomato PME (*14*, *27*, *28*). For example, Rodrigo et al. (*15*) studied the thermal stability of the main PME isoenzyme purified from four tomato varieties, including Perfectpeel, reporting that it could be thermally inactivated at 70 °C for 5 min. With regard to the study of thermal stability of different isoenzymes of tomato PME, there is scarce information. Warrillow et al. (*11*) investigated the effect of temperature on the stability of three isoenzymes of tomato PME. They observed that the temperatures at which 50% of the activity was recovered were 63, 64, and 55 °C. These results are quite in accordance with those found in the present work. However, they did not generate kinetic data.

The thermal inactivation of $CE_{2.3}$ and $CE_{1.1}$ at pH 6.0 could be accurately described by a first-order model in the temperature range studied (**Figure 6A** and **B**, respectively). Their corresponding estimated kinetic parameters are reported in **Table 3**. $CE_{2.3}$ was more thermolabile since only 8 min at 55 °C was sufficient to obtain one log unit reduction of its activity, whereas for $CE_{1.1}$, 31 min at 65 °C was necessary to obtain a similar reduction in enzyme activity. These results confirm the important difference in thermal stability observed between both isoenzymes. **Figure 7** shows that the thermal inactivation of $CE_{2.3}$ was less temperature-sensitive than for $CE_{1.1}$, as indicated by Z_T values of 6.6 and 4.9 °C, respectively. These values are in the same range as those reported by others authors for the main

Table 3. Estimated Kinetic Parameters for Thermal Inactivation of $CE_{2.3}$ and $CE_{1.1}$ in 0.10 M Citric Acid Buffer at pH 6.0[a]

T (°C)	$D(CE_{2.3})$ (min)	$D(CE_{1.1})$ (min)
51	34.83 ± 1.01[b]	nd
53	17.75 ± 0.32	nd
55	8.38 ± 0.28	nd
57	3.85 ± 0.23	nd
63	nd	73.42 ± 2.82
65	nd	30.76 ± 1.01
67	nd	10.26 ± 0.21
69	nd	4.16 ± 0.15
Z_T (°C)	6.62 ± 0.20	4.93 ± 0.19
95% confidence interval	[6.20, 7.04]	[4.55, 5.31]

[a] nd: not determined. [b] Standard error of regression.

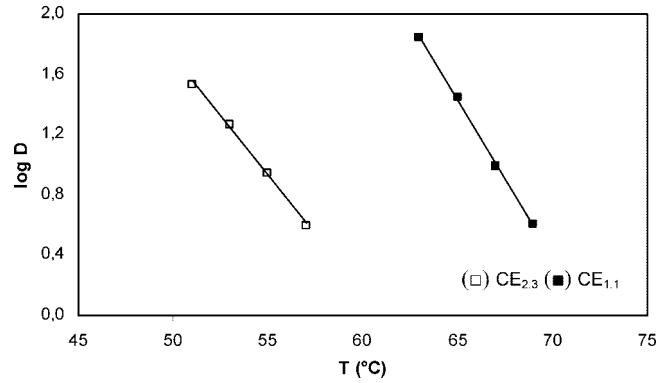

Figure 7. Temperature dependence of the decimal reduction time for thermal inactivation of purified tomato PME in 0.10 M citric acid buffer at pH 6.0.

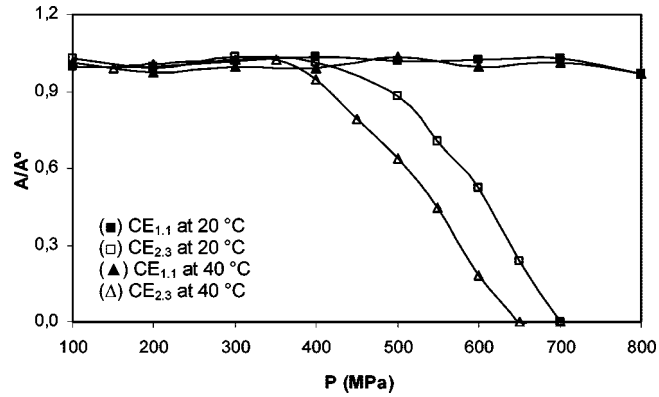

Figure 8. Residual activity of purified tomato PME at pH 6.0 after 5.0 min of treatment at preset pressures.

isoenzyme (*18*). On the other hand, Laratta et al. (*17*) studied the thermal stability of three forms of PME purified from tomatos obtaining Z_T values of 24, 15, and 24 °C. This is the only work available in the literature that presents kinetic data for the thermal inactivation of tomato PME isoenzymes. The differences observed between both studies could be due to the fact that isoenzymes were obtained from different tomato varieties with different degrees of ripeness, the medium pH, and the procedure used for extraction and purification.

High-Pressure Inactivation of Tomato PME. The high-pressure stability of two major isoenzymes of tomato PME, $CE_{1.1}$ and $CE_{2.3}$, was screened at pH 6.0. Samples were treated at 20.0 and 40.0 °C for 5.0 min in a pressure range from 100 to 800 MPa. A striking difference between the pressure stabilities of both isoenzymes was observed. As shown in **Figure 8**, the thermostable isoenzyme ($CE_{1.1}$) was also found to be pressure-stable since no inactivation was observed after 5.0 min of

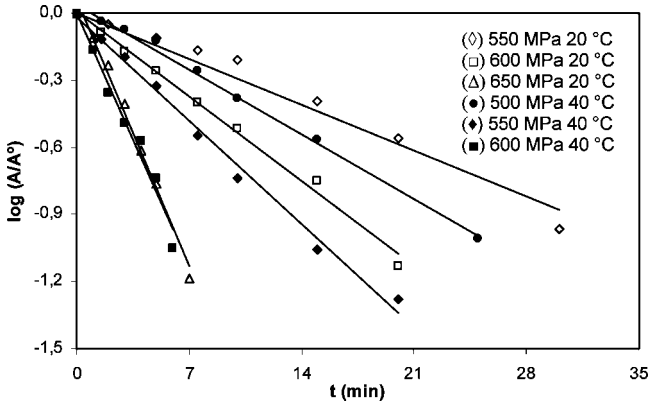

Figure 9. High-pressure inactivation of $CE_{2.3}$ in 0.10 M citric acid buffer at pH 6.0.

Table 4. Estimated Kinetic Parameters for High-Pressure Inactivation of $CE_{2.3}$ at 20 and 40 °C in 0.10 M Citric Acid Buffer at pH 6.0[a]

P (MPa)	D_{20} (min)	D_{40} (min)
500	nd	24.21 ± 0.74[b]
550	31.43 ± 1.70	15.11 ± 0.55
600	18.45 ± 0.63	6.20 ± 0.45
650	5.85 ± 0.28	nd
Z_P (MPa)	135.90 ± 5.29	149.70 ± 6.07
95% confidence interval	[124.90, 146.90]	[137.10, 162.40]

[a] nd: not determined. [b] Standard error of regression.

treatment at 800 MPa and 20 or 40 °C. On the other hand, the thermolabile isoenzyme ($CE_{2.3}$) appeared to be pressure labile since it could be completely inactivated after 5.0 min of treatment at 700 MPa and 20 °C or 650 MPa and 40 °C. Although other authors have also reported that the main isoenzyme was pressure-stable (*16*, *29*), this is the first time that a pressure labile PME isoenzyme is reported in tomato fruit. With regard to other sources, Ly-Nguyen et al. (*30*) studied the high-pressure inactivation of purified carrot PME. They observed the presence of a pressure-stable fraction (more or less 3% of the total activity) and the occurrence of a pressure-sensitive carrot PME fraction, which could be inactivated in the pressure range of 600–700 MPa after treatment for more than 60 min. Also, Guiavarc'h et al. (*31*) found a pressure labile PME fraction in grapefruit. However, taking into account the available literature, so far, the PME isoenzyme most sensitive to pressure is that reported by us in the present work.

As was the case for thermal inactivation, high-pressure inactivation of $CE_{2.3}$ at pH 6.0 could be accurately described by a first-order model in the pressure range studied (**Figure 9**). **Table 4** shows the estimated kinetic parameters. $CE_{2.3}$ was more pressure labile at 40 °C since only 6 min at 600 MPa was required to obtain one log unit reduction of its activity, whereas for the same inactivation level at 20 °C, 18 min at 600 MPa was necessary. As shown in **Figure 10**, the high-pressure inactivation of $CE_{2.3}$ at 20 °C was slightly more pressure-sensitive than at 40 °C, as indicated by Z_P values of 135.9 and 149.7 MPa, respectively. On the contrary, Balogh et al. (*32*) studied the high-pressure inactivation of purified carrot PME (pH 6) at 10 and 25 °C, reporting that it was slightly less pressure-sensitive at lower temperatures. Therefore, depending on the source of PME, different kinetic properties can be observed.

The effect of PME on the texture of (processed) tomato products cannot be explained by studying a single isoenzyme since several isoenzymes with different processing stabilities are involved. Kinetic data are of key importance for the implementation and optimization of thermal and high-pressure processing of tomato products.

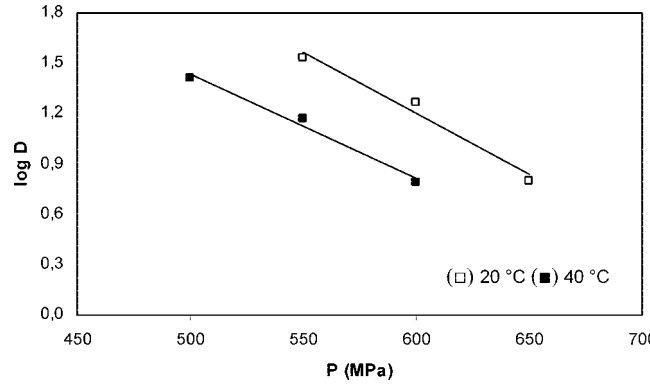

Figure 10. Pressure dependence of the decimal reduction time for high-pressure inactivation of $CE_{2.3}$ in 0.10 M citric acid buffer at pH 6.0.

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