Thermal Inactivation of Pectin Methylesterase, Polygalacturonase, and Peroxidase in Tomato Juice

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Thermal inactivation kinetics have been determined for pectin methylesterase (PME), polygalacturonase (PG), and peroxidase (POD) in tomato juice. Two parameters, the inactivation rate constant (k) at a reference temperature and the activation energy for inactivation (E_a), were determined for each enzyme. For PME and PG, the k and E_a values reported here do not agree with those in several previously published reports. These differences can be explained either by the differences in pH values used for inactivation determinations or by inadequacies in the heating methods used in some previous studies. POD showed simple first-order inactivation kinetics and was less thermally stable than either PME or PG. When different cultivars of tomatoes were evaluated, there was no difference in the thermal inactivation kinetics of these enzymes.

INTRODUCTION

Tomatoes are thermally processed to make a number of different products such as juice, soup, ketchup, and other sauces. The desired final consistency of each of these products varies. For products such as soup and juice, good retention of color and flavor are important, as is a lower viscosity. In other products such as ketchup, pizza, and spaghetti sauces, a greater final viscosity is desired. The viscosity of tomato products is strongly affected by the composition of the pectins. Controlling the breakdown or retention of the pectins, and the enzymes that lead to changes in the pectins, is thus of great importance during processing (*1*, *2*).

Pectins in tomatoes consists of a polygalacturonic acid backbone with a variable number of methyl groups esterified to the galacturonic acid residues. Two enzymes, pectin methylesterase (PME) and polygalcturonase (PG), are involved in the breakdown of these pectins. PME catalyzes the removal of the methyl groups from the polygalacturonic acid chain, leaving an increased number of free carboxyl groups that can then bind cations and cross-link the pectin chains. This cross-linking of the pectin is desirable in particulate products such as diced tomatoes, where it leads to better integrity in the dice and a firmer texture. In juices, it is generally undesirable because the cross-linked pectins can aggregate and settle, leading to a loss of juice cloud. The action of PME also makes the pectin susceptible to further degradation by PG because this enzyme acts only on segments of the pectin chain that have been demethylated by PME. PG cleaves the polygalacturonic acid backbone of the pectin and reduces the average length of the pectin chains. This degradation of the pectin chains reduces the viscosity of the juice.

Two different process are commonly used in the production of tomato paste. In the "hot-break" process, tomatoes are rapidly heated to 95 °C immediately after homogenization. This process is believed to inactivate enzymes rapidly, particularly those involved in pectin degradation, and gives a product with high viscosity (*3*). In the "cold-break" process, the homogenized tomatoes are heated only to around 60 °C. This is believed to have several benefits in the production of products such as juice and soup. The lower temperature reduces the amount of thermal abuse of the product, giving a greater retention of color and flavor components and reducing production of undesirable compounds. The lower temperature also does not entirely inactivate the enzymes PME and PG and allows these enzymes to break down of some of the pectins reducing the viscosity of the juice, favorably affect pumping and evaporation of the juice (*1*).

A fine-tuning of the hot- and cold-break processes requires reliable information on the stability of the relevant enzymes in tomato juice. Current information on the inactivation of these enzymes in tomatoes is incomplete. There are several reports on the kinetics of PME inactivation (*4*–*8*), but the inactivation parameters reported vary significantly. In some cases, inactivation measurements were made with an extracted enzyme suspended in buffer, a method known to give results that differ from those obtained for an enzyme in a crude homogenate. In the two cases where inactivation was measured in tomato juice, there is a lack of agreement in the reported results. Thermal

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inactivation of PG in both the buffer and crude juice have also been reported (*4, 6*), but again the reported results do not agree.

Given the lack of agreement between existing reports for the inactivation of PME and PG, we have undertaken an examination of the inactivation of both of these enzymes in tomato juice. We have also investigated the inactivation kinetics of peroxidase. Whereas POD may not have any direct role in tomato quality, this enzyme is a commonly used indicator of enzyme inactivation in many fruits and vegetables because of its high thermal stability and its ease of assay. There is currently no published data for the thermal inactivation of POD in tomatoes. The inactivation kinetics for all three enzymes were determined in two different cultivars of tomatoes, one of which is primarily used in hot-break processing and the other, in cold-break processing, to determine if differences in enzyme stabilities exist between these cultivars.

MATERIALS AND METHODS

Tomatoes. Field-grown tomatoes of the cultivars BOS 3155 (Orsetti Seed Co., Hollister, CA), which is used in hot-break processing, and CXD-199 (Campbell's Seed Supply Co., Davis, CA), which is used primarily for cold-break processing, were obtained from Campbells Research Center in Davis, CA. Tomatoes of other hot- and cold-break cultivars commonly grown in California such as Heinz 9492 and CXD 152 as well as several Japanese cultivars were also examined, but the details of their inactivation kinetics are not presented here. Tomatoes were washed, sliced in half to remove the seeds and locular gel, and then cut into half-inch dices. The diced tomatoes were frozen in a -80 °C blast freezer and then stored in polyethylene bags at -20 °C or colder until used.

Heating of Homogenates. Homogenates were prepared by grinding 100 g of thawed diced tomatoes for 30 s in a small Waring blender. The homogenate was then passed through a fine metal screen to remove pieces of skin and any seeds. Using a syringe with a blunt needle, we transferred the homogenate to glass tubes that were then heated for the times and temperatures as indicated, cooled in ice water, and held on ice until assay.

The glass tubes used for heat inactivation, which were open at one end and sealed at the other, had a capacity of either 200 μL (1.5-mm i.d. × 2.3-mm o.d. × 125-mm length) or 1.0 mL (4.8-mm i.d × 7.0-mm o.d. × 105-mm length). Come-up times, the times necessary for the solution at the center of the tube to reach the temperature of the surrounding water bath, were determined with a fine thermal probe placed at the center of the tube. For the 200-μL capillary tubes, this come-up time was 10 s, with 90% of the final temperature change achieved in only 5 s. In the 1-mL TDT tubes, come-up times increased to 60 s with 90% of the temperature change occurring in 30 s. Despite the longer come-up times, the larger-capacity tubes were used for PME and PG inactivation determinations because of the amount of material needed to assay these enzymes. When time courses shorter than 1 min were performed, multiple 200-μL tubes were used, and their contents were pooled.

PME Activity Measurement. PME activity was determined titrimetrically following the procedure described in (*9*). A 30-mL aliquot of a solution containing 0.2 M NaCl and 1.0% pectin (P9135, from citrus, Sigma, St. Louis, MO) was equilibrated to 30 °C and adjusted to pH 7.0. Following the addition of 1.0 mL of the heated homogenate, the pH was readjusted to 7.0 and maintained at this pH for 10 min by the addition of either 0.05 or 0.005 N NaOH, depending on the activity of the sample. The rate was calculated as μmol of NaOH consumed over the 10-min time course. The rate of NaOH consumption by a sample of boiled (20 min) homogenate was subtracted as a blank. All activities for heat treatments are reported as percentages of the activities obtained for unheated controls, which ranged between 115 and 135 μmol H$^+$ min^{-1} mL^{-1}.

PG Activity. A crude tomato homogenate cannot be assayed directly for PG activity because of the presence of endogenous reducing sugars and other interfering material. To clean up the heated samples of homogenate to permit assay, the procedure described by Pressey was

used (*10*). A total of 5 mL of heated homogenate from five 1.0-mL TDT tubes was transferred to a 50-mL centrifuge tube, and 10 mL of cold water was added. The pH was adjusted to 3.0 with 0.1 N HCl, and then the sample was centrifuged for 15 min at 10000*g*. The pellet was resuspended in a total of 15 mL of water and homogenized with a polytron. This was centrifuged as before, and this pellet was resuspended in 7.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) containing either 0.5 M NaCl, for extraction of PG2, or 1.2 M NaCl, for extraction of PG1. After 30 min on ice, the samples were centrifuged as before, and the supernatant was collected for assay.

The high salt extracts of the heated homogenates were assayed for PG activity by measuring the production of reducing sugars from polygalacturonic acid. A 1% stock solution of polygalacturonic acid was prepared by dissolving the polygalcturonic acid in 50 mM citrate buffer and adjusting the pH to 4.5. The enzyme incubations contained 0.5 mL of this stock solution and 0.2 mL of 1 M NaCl in a final volume of 2.0 mL to give final concentrations of 0.25% polygalacturonic acid and 0.1 M NaCl. Incubations were started by the addition of 0.2 mL of the high salt extracts and placed in a 37 °C water bath for 30 min. A blank was prepared by boiling an aliquot of the salt-extracted enzyme for 15 min and then incubating in the same manner as for the samples.

Reactions were terminated by the addition of 5.0 mL of the carbonate buffer, described below, and an aliquot of the terminated reaction was analyzed for reducing sugars colorimetrically (*11*). Reagents for this procedure were carbonate buffer containing 54.3 g/L Na$_2$CO$_3$ and 24.2 g/L NaHCO$_3$; reagent A prepared by dissolving 0.98 g of bicinchoninic acid, sodium salt (Pierce, Rockford, IL) in 0.5 L of the carbonate buffer; and reagent B containing 1.24 g/L CuSO$_4$·5H$_2$O and 1.26 g/L L-serine. A working reagent was prepared by mixing equal volumes of A and B immediately prior to use. For the determination of reducing sugars, 0.6 mL of the terminated enzyme incubations were mixed with1.6 mL of this working reagent and 1.9 mL of water. Samples were heated for 30 min at 80 °C and then allowed to cool to room temperature, and absorbance at 560 nm was determined. Activity was calculated on the basis of the boiled enzyme blank and a standard curve of galacturonic acid.

POD Activity. Following heat treatment in a pair of 200-μL capillary tubes, samples were prepared for assay by mixing 0.4 mL of heated homogenate with 0.1 mL of 0.5 M phosphate buffer, pH 6.5, in a 1.5-mL microcentrifuge tube. Samples were centrifuged for 10 min at 10000*g*, and the pellet was discarded. The supernatant was desalted by centrifuging through a column (2.0-mL bed volume) of Sephadex G-25 equilibrated with 0.1 M phosphate buffer, pH 6.5 (*12*).

Alternatively, in one experiment, this centrifugation and desalting procedure was performed prior to the heating step. In this case, tomatoes were homogenized in an equal volume of 0.1 M phosphate buffer, filtered through cheesecloth, and centrifuged. A 1.0-mL aliquot of the supernatant was desalted as described above except a 6.0-mL bed volume was used. This desalted supernatant was then heated as indicated in 20-μL capillary tubes and assayed for residual POD activity.

POD activity was determined as described (*13*). The 1-mL assay volume contained 50 mM phosphate buffer (pH 6.0), 5 mM dimethylaminobenzoic acid, 0.1 mM EDTA, 0.1 mM 3-methyl-2-benzothiazolinone hydrozone (MBTH), and 10 mM H$_2$O$_2$. Reactions were started by the addition of 50 μL of heated homogenate, and the absorbance increase at 590 nm was monitored for up to 10 min, with the slope of the linear portion of the curve used to determine activity. EDTA, which had no effect on peroxidase acticvity, was included in the peroxidase assay to ensure that nonenzymatic color formation, catalyzed by a trace amount of Fe or Cu, did not occur.

Calculations. The rate constants *k* for first-order inactivation were determined from the slopes of the inactivation time courses according to eq 1

$$\log (A/A_0) = -(k/2.303)t \qquad (1)$$

where A_0 is the initial enzyme activity and *A* is the activity after heating for time *t*. The slopes of these lines were determined by linear regression, and the calculated rate constants were replotted in Arrhenius plots. Standard errors for the rate constants, calculated from the regression lines of the inactivation data, are shown as error bars on the

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data points in the Arrhenius plots. In most cases, these error bars are smaller than the symbol size. Activation energies (E_a) were calculated from the slopes of the Arrhenius plots of $\ln(k)$ versus $1/T$ according to eq 2

$$\ln(k) = -E_a/RT + c \qquad (2)$$

where R is the gas constant (8.314 J mol^{-1} K^{-1}) and T is the temperature in K. Slopes and their standard errors were calculated by linear regression.

For each enzyme, a reference temperature was chosen in the temperature range used for inactivation determinations. The rate constant for inactivation at this reference temperature (k_{ref}) was determined from the value of $\ln(k_{ref})$ at this temperature given by the regression line in the Arrhenius plot. Error estimates for k_{ref} were estimated on the basis of the error estimates determined for $\ln(k_{ref})$ obtained from this regression line. Converting the error in $\ln(k_{ref})$ to an error in k_{ref}, however, gives asymmetric estimates for the error in k_{ref}. The error we report is the larger of these two estimates. With the two parameters E_a and k_{ref}, the rate of inactivation (k) at any temperature (T) can be calculated from eq 3.

$$\ln(k) = \ln(k_{ref}) - (E_a/R)(1/T - 1/T_{ref}) \qquad (3)$$

We have expressed our inactivation data in terms of the two parameters k and E_a. Much of the previously published data for the inactivation of PME and PG in tomatoes is expressed as D and z values. To interconvert these two sets of parameters, the following calculations were used. A D value, the time required to reduce the enzyme activity to 10% of its original value, is directly related to an inactivation rate constant k by eq 4:

$$k = 2.303/D \qquad (4)$$

A z value and an E_a value are related as follows. It can be shown by substituting eq 4 into eq 2, changing from natural to base 10 logarithms, and combining all constant terms into a new constant c' that the following is true:

$$\log(D) = E_a/2.303RT + c' \qquad (5)$$

log D is thus linearly related to the *inverse* of temperature, and a plot of log D versus T should be curved. This is inconsistent with the experimental observation that a plot of log D versus T gives an apparently straight line with a slope of $-1/z$. This inconsistency can be reconciled by the fact that in the temperature range typically used for enzyme inactivation, 323 to 373 K (50 to 100 °C), a plot of the inverse of the absolute temperature versus the absolute temperature is very nearly linear. The slight curvature that should be present in the plot of log D versus T is thus insignificant when compared to the experimental scatter in the data.

Even if the relationship between log D and T is not actually linear, the slope of this plot at any given T can still be defined as $-1/z$. This slope is also given by the derivative of eq 5 with respect to temperature:

$$-1/z = d(\log D)/dT = -E_a/2.303RT^2 \qquad (6)$$

E_a is thus related to z according to eq 7, where T(K) is the temperature used for inactivation.

$$E_a = 2.303RT^2/z \qquad (7)$$

Because a range of temperatures must be used to determine a z value, we have taken the median temperature used in z-value determinations to calculate the equivalent E_a for a given z.

RESULTS AND DISCUSSION

PME. Thermal inactivation of PME in the juice of CXD 199 tomatoes showed apparent first-order kinetics. The plot of the log of residual activity versus heating time could reasonably be fit to a straight line over most of the time course (**Figure 1**).
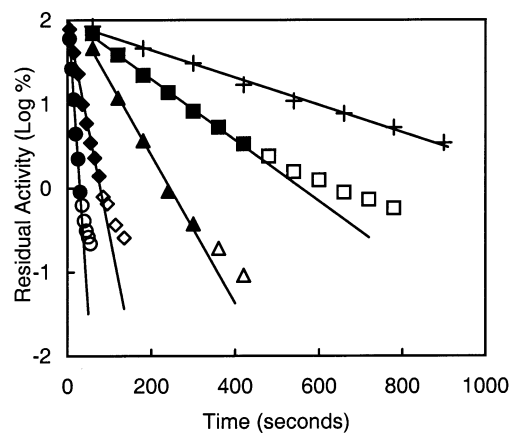

Figure 1. Thermal inactivation of PME. Juice from CXD 199 tomatoes was heated for the indicated times to 69.8 (+), 71.8 (■), 73.8 (▲), 75.8 (◆), or 77.8 °C (●). Open symbols at the four highest temperatures indicate data points not used for the linear regression. For the two highest temperatures, heating was carried out in 200-μL capillary tubes; the other three were heated in 1.0-mL tubes.
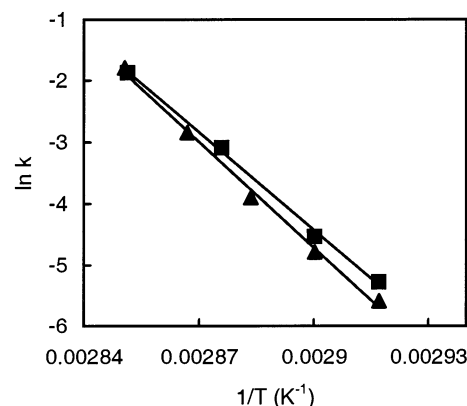

Figure 2. Arrhenius plot of the thermal inactivation rates for PME in CXD 199 (▲) and BOS 3155 (■) tomatoes.

Similar first-order kinetics were obtained for the inactivation of several other tomato cultivars (data not shown). At the longest heating times, there is an apparent deviation from linearity. This deviation could indicate the presence of a second minor isoform of PME with greater thermal resistance. It also could be explained by some subtle assay artifact such as a small systematic error in our determination of the background rate of H$^+$ production in our samples. This latter alternative has to be considered given the nonspecific nature of this assay (H$^+$ production) and the crude nature of our samples. In any case, this deviation is small and apparent only after more than 95% of the activity has been inactivated. It is thus of very minor consequence in predicting the loss of total activity during heating.

The thermal inactivation data in **Figure 1** gave a simple, linear Arrhenius plot (**Figure 2**). The plot of the inactivation data for a second tomato variety, BOS 3155, was very similar. There was, therefore, no difference in the inactivation kinetics between the typical hot-break and cold-break cultivars. The activation energies as well as other inactivation parameters for PME in these two tomato cultivars are listed in **Table 1**. Similar inactivation parameters were determined with juice from several other hot- and cold-break tomato cultivars (data not shown).

Two other groups have determined thermal inactivation kinetics for PME in tomato juice. The results of Crelier et al.

Table 1. Summary of Thermal Inactivation Parameters for Tomato Enzymes[a,b]

| enzyme	tomato cultivar	T_{ref} (°C)	$k_{ref} \times 10^3$ (s^{-1})	E (kJ/mol)	$D_{T_{ref}}$ (min)	z (°C)
PME	CXD 199	70	3.7 ± 0.3	477 ± 19	10.4	4.8
	BOS 3155	70	5.3 ± 0.4	440 ± 15	7.2	5.2
PG1	CXD 199	90	2.4 ± 0.1	324 ± 17	16.1	7.7
	BOS 3155	90	2.4 ± 0.2	351 ± 22	15.8	7.1
PG2	CXD 199	70	14.7 ± 0.4	208 ± 9	2.6	10.8
	BOS 3155	70	9.7 ± 0.3	215 ± 11	4.0	10.4
POD	CXD 199	70	26.1 ± 2.5	546 ± 36	1.5	4.1
	BOS 3155	70	32.4 ± 3.9	557 ± 45	1.2	4.0

[a] Inactivation rates (k_{ref}) at the indicated reference temperatures (T_{ref}), as well as activation energies for inactivation (E_a), were determined from the regression lines fitted to the data in the Arrhenius plots (**Figures 2**, **5**, and **7**) [b] $D_{T_{ref}}$ and z values were calculated from the k_{ref} and E_a values, respectively, using the equations given in Materials and Methods.

(*4*) are in good agreement with ours. They reported a simple first-order inactivation, a linear Arrhenius plot, a rate constant for inactivation at 70 °C of 6.25×10^{-3} s^{-1}, and an activation energy of 350 kJ/mol. These values agree quite well with the values determined in the present study (**Table 1**). Their inactivation data also shows a slight deviation from linearity over long heating times.

In contrast, the earlier results of De Sio et al. (*5*) are quite different. They reported a highly nonlinear plot of log D versus temperature. By taking the slope of the tangent to this curve at temperatures above 80 °C, a z value of 27.8 °C was determined. This is equivalent to an activation energy of only 88 kJ/mol, which is far lower than the value reported by Crelier et al. (*4*) or determined here. Both the odd nonlinearity of the log D versus temperature plot as well as the very low apparent activation energy given by DeSio et al. can be explained if it is assumed that they substantially underestimated the rate of inactivation at the high end of their temperature range. This seems quite likely. Their data is derived from very short time courses (30 s or less) and an experimental protocol that involves heating 1.5-mL samples in plastic bags. The come-up time for heating this volume in a plastic bag may be significant, and a failure to account for this would lead to an underestimation of the inactivation rate. This error would be greatest at the highest temperatures because these temperatures involve the shortest time courses.

Inactivation kinetics for tomato PME have also been determined with purified and partially purified enzyme preparations. Lopez et al. (*6*) measured the inactivation of a partially purified preparation in pH 4 citrate buffer containing 0.4 M NaCl. Simple first-order kinetics were obtained with a D value at 70 °C of 1.53 min and a z value of 5 °C. This corresponds to an inactivation rate constant of 0.192 s^{-1} and an activation energy of 452 kJ/mol. This activation energy is approximately the same as what we determined for the inactivation in juice, but the rate constant at 75 °C is 5 times higher. This difference in the inactivation rate is probably the result of the pH difference between their buffer (4.0) and that of the juice we used (4.4). We have found that the rate of inactivation is strongly dependent on pH at pH values between 4 and 5. For example, when tomato juice with a pH of 4.36 was acidified to 4.00 by the addition of HCl, the rate of inactivation at 71.8 °C was increased more than 4-fold (data not shown).

Inactivation kinetics for commercially available purified tomato PME have also been reported (*7*). In this case, the enzyme preparation was resuspended in water, and a temperature
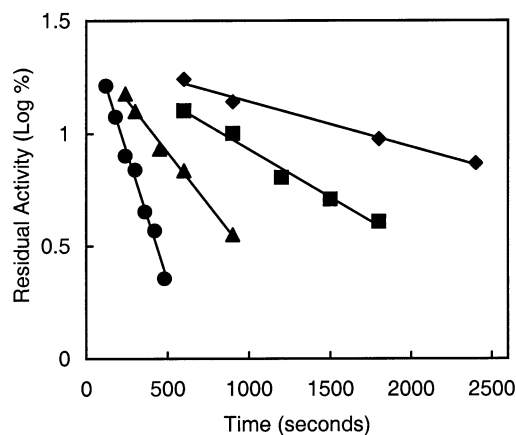
Figure 3. Thermal inactivation of PG1. Juice from CXD 199 tomatoes was heated for the indicated times to 84.8 (◆), 86.7 (■), 89.6 (▲), or 92.8 °C (●).

range of 57 to 65 °C was used for inactivation. Simple first-order kinetics were obtained with a z value of 6.5 °C. This corresponds to an activation energy of 326 kJ/mol, which is similar to that obtained in the present study and by others. By extrapolating these results to 70 °C, a D value of 0.38 min, corresponding to a rate constant of 0.10 s^{-1}, is obtained. This value is 4 times higher than that reported by Lopez et al. (*6*) and more than 20 times greater than that reported in the present study or by Crelier et al. (*4*) for PME in juice. Because these authors do not specify the pH of the resuspended enzyme (it is presumably near 7), it is unknown whether the more rapid inactivation they observed is due to a pH effect or to the lack of other components in the enzyme solution.

The same group that produced the anomalous PME inactivation kinetics in tomato juice has also determined inactivation kinetics for purified PME isozymes, in this case using pH 7.5 buffer and heating in plastic tubes (*8*). They determined z values of between 15 and 24 °C (E_a values of 96 to 155 kJ/mol), which are much lower than what others have determined for purified tomato PME. As described above, the use of plastic vessels for heating and a failure to account for come-up time may account for the anomalous results obtained by this group. The relevance of this inactivation data in pH 7.5 buffer to tomato juice, which typically has a pH of 4.2 to 4.6, is also questionable.

PG. It is well established that there are two forms of PG in tomatoes, designated PG1 and PG2, that differ substantially in their thermal stability. This large difference in thermal stability makes it possible to determine the inactivation kinetics of each independently of the other, even in a crude homogenate, by using the procedure described by Ling and Lund (*14*). The inactivation of PG1 and PG2 in juice from the CXD 199 cultivar is shown in **Figures 3** and **4**. The more heat resistant form, PG1, which comprised about 15% of the total activity, required temperatures of 85 °C and higher for a significant rate of inactivation. PG 2, however, was inactivated rapidly at temperatures below 75 °C. The Arrhenius plot (**Figure 5**) for PG1 showed no difference between the two cultivars of tomatoes and only a small difference for PG2. Inactivation parameters for PG1 and PG2 for both tomato cultivars are given in **Table 1**. There is no apparent difference between the two tomato cultivars except that the rate constant for the inactivation of PG2 at 70 °C was slightly higher for CXD 199 than for BOS 3155. Similar inactivation kinetics were determined for several additional hot- and cold-break tomato cultivars (data not shown).

Thermal inactivation kinetics for tomato PG have been reported by others for both purified PG and for PG in juice.
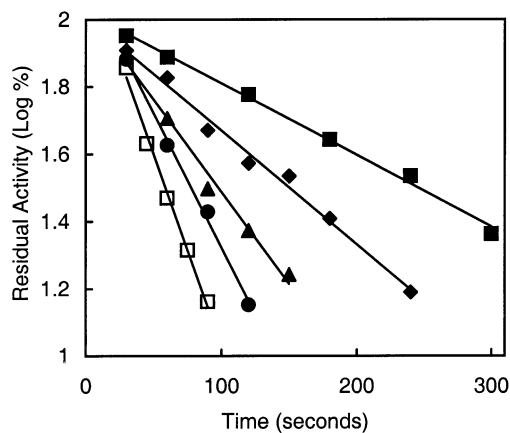
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Figure 4. Thermal inactivation of PG2. Juice from CXD 199 tomatoes was heated for the indicated times to 64.9 (■), 67.0 (◆), 68.9 (▲), 70.9 (●), or 72.8 °C (□).
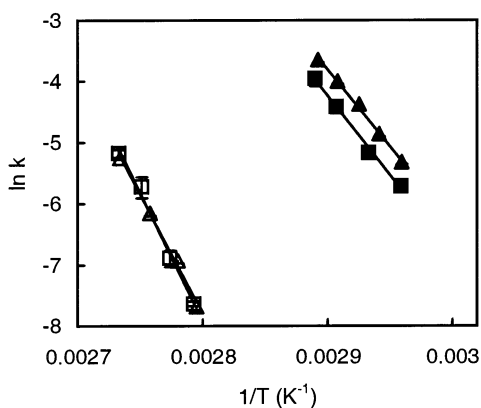

Figure 6. Thermal inactivation of POD. Juice from CXD 199 tomatoes was heated for the indicated times to 66.0 (◆), 68.0 (●), 70.0 (▲), or 72.0 °C (■).

Figure 5. Arrhenius plot of thermal inactivation rates for PG1 (open symbols) and PG2 (closed symbols) in CXD 199 (▲, △) and BOS 3155 (■, □) tomatoes.

Figure 7. Arrhenius plot of thermal inactivation rates for POD. Inactivation was determined in juice with CXD 199 (●), in juice in with BOS 3155 (▲), or in pH 6.5 buffer with BOS 3155 (△) tomatoes.

The existing data for inactivation in juice (*4*) is difficult to interpret because these authors made no distinction between PG1 and PG2. From the temperature range employed, it appears that they were measuring the inactivation of PG1. They were able to model the inactivation reasonably well by assuming simple first-order kinetics of a single isozyme. The relative amounts of PG1 and PG2 in a tomato homogenate are known to be variable (*10*), so it is possible that the juice they were using contained a much higher proportion of PG1 than that used in our study. They reported an inactivation rate constant at 90 °C of 0.0031 s^{-1} and an activation energy of 134 kJ/mol. This rate constant is similar to what we determined at 90 °C; the activation energy is much lower. This difference could arise from their failure to distinguish clearly between PG1 and PG2.

Thermal inactivation kinetics have also been determined for partially purified PG1 and PG2 in pH 4 citrate buffer (*6*). The *z* values of 5.6 and 9.4 °C obtained, corresponding to activation energies of 456 and 238 kJ/mol for PG1 and PG2, respectively, are in reasonable agreement with the values we determined (**Table 1**). Their reported rates of inactivation, however, are higher than ours. At 90 °C, their rate constant for the inactivation of PG1 can be calculated from their reported *D* values to be 0.009 s^{-1}. This is almost 4 times higher than what we found. Similarly, their rate of inactivation of PG2 at 70 °C is 5 to 7 times greater than ours. This difference may be due to the differences in the composition and pH of the buffered medium they used for heating as compared to those of juice. As with
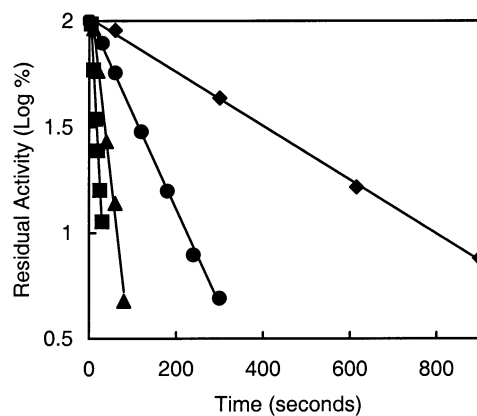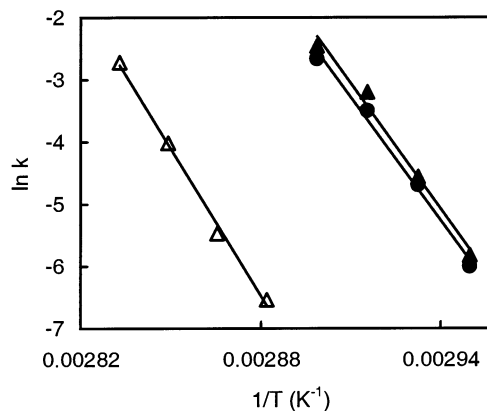
PME, the inactivation kinetics determined in pH 4 buffer give similar activation energies for inactivation to those determined in juice but higher rates of inactivation.

POD. Peroxidase is generally one of the most thermally stable enzymes found in fruits and vegetables. Although the role of POD in causing quality changes is not well established, it is a commonly used indicator for the inactivation of endogenous enzymes during heating because the assay is simple and rapid. Inactivation kinetics for POD from tomatoes have not been previously reported. We investigated the thermal stability of POD to determine whether it would be suitable as a proxy for the inactivation of either PME or PG.

Thermal inactivation of POD in tomato juice showed simple first-order kinetics (**Figure 6**). Arrhenius plots constructed from these inactivation kinetics were also linear and showed little difference between the two cultivars of tomatoes studied (**Figure 7**). Activation energies and rates of inactivation at a reference temperature are given in **Table 1**. These parameters indicate that POD in tomatoes is not especially stable toward heat and would be inactivated more rapidly than PME or either of the PG isoforms. When inactivation was performed on a juice sample that had been passed through Sephadex G-25 equilibrated with pH 6.0 phosphate buffer, first-order kinetics were still obtained, but a higher range of temperatures was required for inactivation. The Arrhenius plot for this desalted preparation was linear, with a slope similar to that obtained for the samples heated in juice. The calculated activation energy for inactivation

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was 657 kJ/mol as compared to 556 kJ/mol for the enzyme in juice. The value of the rate constant for inactivation at the reference temperature of 70 °C, however, was greatly reduced, from 3.24×10^{-2} to 9.2×10^{-5} s^{-1}. This is consistent with the previous observations with PME and PG, where the principal effect of changes in the pH and composition of the solution surrounding the enzyme is to change the rate of inactivation at a given temperature but not to alter the activation energy for inactivation. Similar results have been reported for the thermal inactivation of lipoxygenase from peas, where changes in pH caused a large change in the inactivation rate by only a small change in the activation energy (*15*).

Simple first-order kinetics are not usually obtained for the inactivation of POD in crude plant homogenates because most plants contain multiple isoforms of POD. Tomato mesocarp has been shown to contain only one isoform of POD (*16, 17*), which would account for the simple kinetics we observed here. In most cases, even purified single isoforms of POD do not show simple, linear first-order kinetics. In this regard, the POD from tomatoes appears to be unusual. The activation energy for the inactivation of this enzyme is also much higher than those reported for many other PODs, which are generally in the range of 80 to 200 kJ/mol (*18−22*). However, we have recently determined the activation energies for the inactivation of POD from such diverse sources as peaches, potatoes, and carrots, and in all cases, we found values of at least 450 kJ/mol (*23*). A higher inactivation energy (322 kJ/mol) was also reported for POD from soybeans (*24*). Most of the lower published values were derived from species of *Brassica*.

CONCLUSIONS

Previously reported results on the thermal inactivation of PME in tomatoes were not in agreement with each other. We believe that our results, which agree quite well with the recent report of Crelier et al. (*4*), more accurately reflect the true inactivation of PME in tomato juice. The discrepancies in earlier reports can be ascribed either to poor experimental design (*5*) or the use of a pH other than that found in tomato juice (*6*). A small change in pH from 4.4 to 4.0 appears to have a significant effect on the inactivation kinetics of PME. Given that the pH of tomatoes used in processing can vary from as low as 4.2 to as high as 5.0 depending on the cultivar, maturity at harvest and other cultural factors and a more detailed examination of the effect of pH on the inactivation of PME would appear to be warranted. The differences between our PG results and those of Lopez et al. (*6*) may indicate that PG inactivation, like PME inactivation, is also sensitive to relatively small changes in pH at values near those normally found in juice.

The thermal stability of POD was less than that of either PG or PME and thus would not be a good indicator for enzyme inactivation in tomatoes. At the same time, compared to peroxidases from other sources, tomato peroxidase appears to have unusually simple inactivation kinetics and may be a useful model for understanding the mechanism of peroxidase inactivation. None of the activities examined showed any obvious difference between the two cultivars of tomatoes examined. Therefore, the differences between tomato cultivars that make them more or less desirable for hot- or cold-break processing do not appear to be due to the thermal stability of the enzymes.

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