

## Kinetic Parameters for the Thermal Inactivation of Quality-Related Enzymes in Carrots and Potatoes

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Kinetic parameters for the thermal inactivation of several enzymes in carrot and potato homogenates have been determined. In carrots the most heat-resistant activity was polygalacturonase, followed by peroxidase and pectinmethylesterase. In potatoes peroxidase was the most resistant, followed by pectin methylesterase, polyphenol oxidase, and lipoxygenase. There were several notable similarities between the inactivation kinetics in the two vegetables. In both cases peroxidase activity gave simple first-order inactivation kinetics but yielded a curved Arrhenius plot for the temperature dependence. Pectin methylesterase in both commodities consisted of a labile and a resistant form. The relative amounts of the two forms and the temperature dependences for their inactivation were also similar.

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**KEYWORDS:** Thermal inactivation; kinetic parameters; peroxidase; pectin methylesterase; polygalacturonase; carrots; potatoes

### INTRODUCTION

It is well-known that the presence of residual endogenous enzymes in either raw or processed fruit and vegetable products may cause a loss of quality during storage. These changes can affect the texture, color, flavor, and nutritional quality of the product (1). Some examples of this include the enzymes polygalacturonase (PG) and pectin methylesterase (PME), which are involved in the degradation of pectins and therefore affect product viscosity and texture. PME activity has been shown to cause cloud loss in juices (2). PG activity may further contribute to the degradation of pectin, leading to thinning of purees and loss of particulate texture (3). Other examples of enzymes that affect quality include lipoxygenase (LOX), which has been shown to be a primary contributor to off-flavor development in a number of commodities (4), and polyphenol oxidase (PPO or catechol oxidase), which is responsible for browning and off-color development (5).

To prevent unwanted changes during storage, fruit and vegetable products are generally subjected to some type of treatment during processing in order to inactivate these enzymes. A heat treatment, such as blanching, pasteurization, or commercial sterilization, is most commonly used; however, other processes such as high pressure or pulsed electric fields have also been proposed. The effectiveness of these other processes on enzyme inactivation is controversial (6, 7). Heat treatment of vegetables can also lead to the loss of desirable characteristics such as color, texture, flavor, and nutrients such as ascorbic acid. For this reason it is desirable to keep the heat treatment to a minimum yet still have it be sufficient to completely inactivate the deleterious enzymes. In most cases, complete enzyme inactivation is the target; there is very little information

available in the literature on the effects of low levels of residual enzymes on stored product quality.

To monitor the heat treatment, an indicator enzyme is often assayed during the process to assess the degree of inactivation. Although its role in causing quality problems during storage is controversial (4, 8, 9), peroxidase (POD) is usually the indicator enzyme of choice in fruit and vegetable freezing operations because of its high concentration in most plant tissues, its high thermal stability, and its ease of assay. The high thermal stability of POD can be seen as either an advantage or a problem. On the one hand, it provides a natural margin of safety in that if peroxidase is inactivated, it is a reasonable assumption that other quality-related enzymes have also been inactivated. On the other hand, the reliance on peroxidase as an indicator may lead to an excessive heat treatment of the product and cause other quality problems. Lipoxygenase has been proposed as an alternative indicator enzyme for some commodities (4). Preservation methods other than freezing, such as juice processing, canning, and dehydration, typically do not use enzyme indicators; rather, they target microbial inactivation and assume that enzyme inactivation will be accomplished simultaneously.

One approach to optimizing the heat treatment of fruits and vegetables in order to maximize quality is to develop a model incorporating, among many other parameters, the inactivation kinetics for relevant enzymes to predict quality changes during processing and subsequent storage. Two parameters are needed to characterize the thermal stability of a given enzyme. One is the rate of inactivation at a specified temperature, expressed either as a rate constant or as a *D* value, and the other is a measure of how the rate of inactivation varies with temperature, given by either an activation energy ( $E_a$ ) or a *z* value. With these two parameters the rate of enzyme inactivation at any temperature, and accordingly the expected level of residual activity remaining after a given heat treatment, can be calculated.

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If multiple isozymes with differing thermal stabilities are present, then multiple inactivation rates must be determined and the overall level of residual activity may be calculated by summing the residual levels of the individual isozymes. In cases when inactivation does not show simple first-order kinetics or when the variation in the inactivation rate with temperature is complex (nonlinear Arrhenius plots), such simple calculations are not valid, and other parameters or limiting assumptions must be introduced to model the data.

Before a model of quality changes due to processing and storage can be developed, accurate data on the thermal stability of the relevant enzymes must be ascertained. For the two commodities that we have chosen for such modeling, carrots and potatoes, the existing data on thermal inactivation of enzymes is inadequate. The limited published data for the thermal stability of enzymes in carrots is generally of poor quality and not useful for quantitative modeling (10). With the exception of lipoxygenase (11) there are also no thermal inactivation data for quality-related enzymes in potatoes. We report here more detailed inactivation kinetic data for several quality-related enzymes in these two commodities.

## MATERIALS AND METHODS

**Materials.** Chemicals were obtained from Sigma (St. Louis, MO). Potatoes (cv. Russet Burbank) and carrots (unspecified variety) were obtained from a local market.

**Preparation of Homogenates.** Potatoes were washed, peeled, diced into ~1 cm cubes, and stored at -20 °C until use. Minor surface browning occurred on the cubes. Fifty grams of frozen potato was homogenized in 50 mL of 0.1 M phosphate buffer (pH 6) in a Waring blender. The homogenate was filtered through cheesecloth and then centrifuged at 3800g for 5 min. The supernatant was desalted by passage through Sephadex G-25 equilibrated with 0.1 M phosphate buffer (pH 6) using the centrifugation method of Helmerhorst and Stokes (12). This desalting step was included to remove phenolic compounds that caused the potato homogenates to rapidly brown.

Carrot juice was prepared by homogenizing 100 g of peeled carrots in 50 mL of water and then filtering through cheesecloth. This juice was then used for thermal inactivation without further purification. All solutions used in the preparation of the homogenates as well as the plant material were kept at refrigerator temperature or on ice. The homogenates were held on ice prior to heat treatment.

**Thermal Inactivation.** Aliquots of the homogenates were transferred to capillary tubes (1 mm i.d., 200  $\mu$ L total volume) with a syringe and held on ice until heating. Samples were heated in a circulating water bath (model 20B, Julabo, Allentown, PA) to the indicated temperatures (between 60 and 85 °C depending on the enzyme) for the times specified. The temperature of the water bath was verified with a calibrated mercury thermometer (model 1005-3FC, Ertco, West Patterson, NJ) and was controlled to  $\pm 0.1$  °C. A plexiglass cover, with a small hole in it to allow for the insertion and removal of samples from the bath, was employed to prevent evaporative cooling and was necessary to obtain temperatures >80 °C. The come-up time for the capillary tubes was determined by placing a fine thermocouple in the solution at the center of the tube and recording the time necessary for the solution in the tube to reach that of the water bath. This time was determined to be <10 s.

Following heating, samples were cooled in ice water and stored on ice until assay. The potato extracts were assayed for enzyme activities without any further treatment. For the carrot homogenates, only PME could be assayed without further treatment. To assay POD, particulate material was removed by centrifugation for 10 min at 10000g and the supernatant used for the assay. A small portion of the activity (~10%) remained in the pellet. Samples to be assayed for PG required more extensive preparation prior to assay. A portion of the total PG activity in carrots has been shown to require the inclusion of high salt for solubilization (13). Accordingly, samples of heated homogenate (0.4 mL) were mixed with 0.1 mL of 5.0 M NaCl, allowed to stand 10 min

on ice, and then centrifuged for 10 min at 10000g. The supernatants were then desalted by centrifugation through Sephadex G-25 equilibrated with 0.1 M acetate buffer (pH 5) to remove any residual reducing sugars. The presence of even low amounts of reducing sugars in the enzyme preparation caused an unacceptably high background color in the PG assay. To ensure complete removal of the reducing sugars, it was necessary to perform this centrifugal desalting procedure twice.

**Enzyme Assays. Peroxidase.** Activity was measured using the method of Ngo and Lenhoff (14). 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<sub>2</sub>O<sub>2</sub>. Reactions were started by the addition of 20  $\mu$ L of potato or 50  $\mu$ L of carrot homogenate. 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 POD activity, was included in the POD assay to ensure that nonenzymatic color formation, catalyzed by trace amounts of Fe or Cu, did not occur.

**Pectin Methyltransferase.** Activity was quantified as carboxyl groups formed by the hydrolysis of methyl esters of pectin and was measured titrimetrically using a pH electrode to monitor the production of H<sup>+</sup> (15). Specific conditions for the assay were based on those given in ref 16. The reaction contained the following in a final volume of 2.0 mL: 0.25% citrus pectin, 0.25 M NaCl, and 0.1 mL of homogenate. The sample was maintained at 30 °C and the pH monitored with a pH electrode. The pH was carefully adjusted to ~7.2 by small additions of 0.1 and 0.01 N NaOH. The drop in pH due to H<sup>+</sup> generation by PME activity was then followed until the pH reached 7.0. At this point, 2–10  $\mu$ L of 0.01 N NaOH was added, which raised the pH to ~7.5, and the time required to return to pH 7.0 was recorded. This procedure was repeated several times until a stable rate of H<sup>+</sup> generation was observed.

**Polygalacturonase.** Activity was measured by colorimetrically quantifying galacturonic acid residues liberated from polygalacturonic acid by PG. Assay conditions were based of those described in ref 13. Incubations contained 0.3 mL of desalted carrot homogenate in 0.1 M acetate buffer (pH 5) and 0.05 mL of 0.5% polygalacturonic acid and were incubated for 1 h at 37 °C. Samples (0.1 mL) were taken from the incubations, either immediately after mixing (zero time) or after 1 h of incubation. The amount of reducing sugar was determined colorimetrically by reaction with MBTH (17).

**Lipoxygenase.** Lipoxygenase was measured by quantifying at 234 nm the formation of conjugated dienes from linoleic acid (18). The linoleic acid substrate was prepared by mixing 140 mg (155  $\mu$ L) of linoleic acid and 280 mg (257  $\mu$ L) of Tween-20 in 5 mL of water. The solution was clarified by the addition of 0.6 mL of 1 N NaOH, then brought to a final volume of 25 mL, and stored frozen under N<sub>2</sub> in 1.0 mL aliquots. Assays were performed at room temperature in 1.0 mL of a medium containing 100 mM phosphate buffer (pH 6) and 10  $\mu$ L of linoleic acid substrate to give a final linoleic acid concentration of 0.25 mM. Reactions were started by the addition of 10  $\mu$ L of potato homogenate. At times of 15 s, 1 min, or 3 min after the start of the reaction, 50  $\mu$ L of 10% SDS was added to stop the reaction and eliminate turbidity prior to reading the absorbance at 234 nm. The rate was calculated from the change in absorbance at 234 nm over this time span.

**Polyphenol Oxidase.** Activity was measured spectrophotometrically following the method of Espin et al. (19). Assays were performed at room temperature in 1.0 mL of a medium containing 5.0 mM dihydroxyhydrocinnamic acid, 0.2 mM MBTH, 0.1% (w/v) Triton X-100, and 100 mM phosphate buffer (pH 5). Incubations were started by the addition of 10  $\mu$ L of potato homogenate, and the increase in absorbance at 500 nm was monitored for up to 30 s.

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

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

where  $A_0$  is the initial enzyme activity and  $A$  is the activity after heating for time  $t$ . Slopes of these lines were determined by linear regression

and the calculated rate constants 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 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 these Arrhenius plots according to eq 2

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

where  $R$  is the gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ) and  $T$  is the temperature in K. Slopes and their standard errors were calculated by linear regression. When inactivation did not follow simple first-order kinetics, the inactivation curves could be resolved into heat labile and heat stable phases following the procedure outlined by Ling and Lund (20), and two inactivation rate constants determined.

For each enzyme a reference temperature was chosen near the middle of 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 inactivation rate constant ( $k$ ) at any temperature ( $T$ ) can be calculated from eq 3:

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

In some cases inactivation is given as a  $D$  value, the time required to reduce the enzyme activity to 10% of its original value. The  $D$  value is directly related to the inactivation rate constant  $k$  by eq 4:

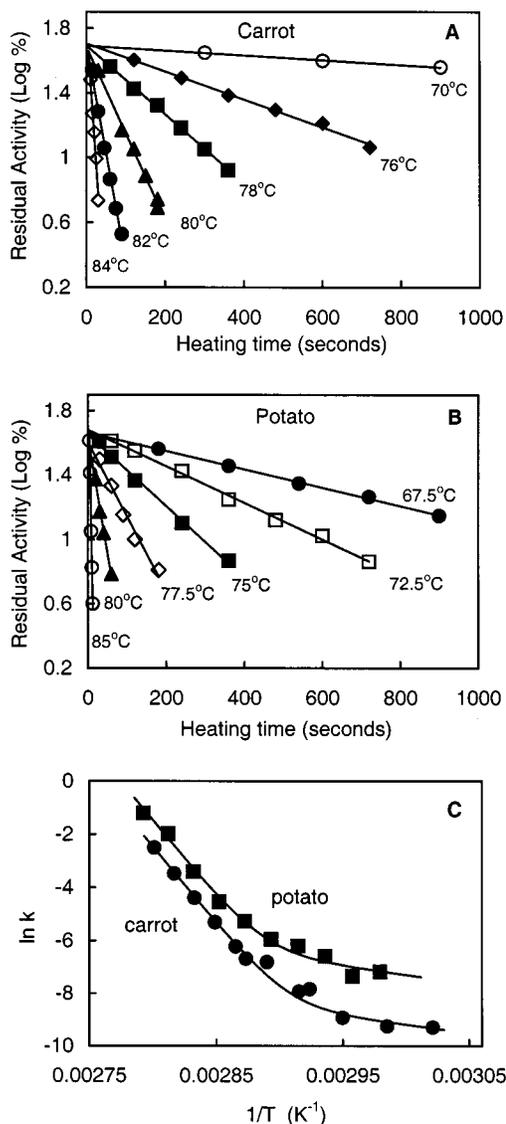
$$D = 2.303/k \quad (4)$$

To compare the relative stabilities of the different enzymes we have calculated, according to eqs 3 and 4, the temperature at which  $D = 5$  min for each enzyme.

## RESULTS AND DISCUSSION

**Peroxidase.** In both carrots and potatoes, about half of the total POD activity was rapidly inactivated at the temperatures studied. This rapid loss of activity is presumably due to the inactivation of a labile isoform of the enzyme. The remaining half of the activity presumably arises from a more resistant form. Labile and resistant forms of POD are known to exist in a number of vegetables (20–22). Guner and Bayindirli (23) reported that 50% of the total POD activity in carrots was heat resistant, consistent with what we report here. Inactivation kinetics for the resistant portion of the activity were determined for both carrots and potatoes. The semilog plots of the residual activity versus heating time were linear at all temperatures studied (Figure 1A,B), consistent with inactivation occurring by a simple first-order process. The fact that the lines all extrapolate back to a common point indicates that the inactivation of the same resistant fraction of the total activity is being measured in each case.

From the slopes of these lines inactivation rate constants were calculated and plotted in an Arrhenius plot (Figure 1C). The Arrhenius plots for both carrots and potatoes show distinct upward curvature. At temperatures  $>78^\circ\text{C}$  (i.e., at  $1/T < 0.00285$ ) the plots can be approximated by straight lines. From the slopes of these lines, activation energies of 480 kJ/mol for carrots and 478 kJ/mol potatoes were calculated. Upward-curving Arrhenius plots have not previously been reported for POD but have recently been noted for lipoxygenase (24, 25). The simplest explanation for such curvature is that inactivation occurs by more than one mechanism, each with its own



**Figure 1.** Heat inactivation of peroxidase in carrot and potato homogenates: (A) residual POD activity versus heating time in carrots; (B) residual activity versus heating time in potatoes; (C) Arrhenius plot of inactivation rates from (A) and (B).

temperature dependence. The observed overall temperature dependence is simply the sum of the individual processes. At temperatures  $>78^\circ\text{C}$ , inactivation is the result of some process, such as protein unfolding, with a high activation energy. The high activation energy necessarily means that the rate of this process is strongly temperature dependent, and thus that at lower temperatures this rate becomes insignificant. At these lower temperatures, then, the observed rate reflects some other process with a much lower activation energy, such as the dissociation of heme or the loss of some other functional group. Both protein denaturation and loss of heme have been shown to be mechanisms by which POD is inactivated (26, 27).

If POD inactivation occurs by two parallel pathways, the rate of inactivation can be written as

$$-dA/dt = k_1[A] + k_2[A] = (k_1 + k_2)[A] \quad (5)$$

where  $k_1$  and  $k_2$  are the rate constants for the two pathways and  $A$  is the total activity level. Inactivation will still follow apparent first-order kinetics with a rate constant equal to the sum of the two individual rate constants. These two rate constants will have

**Table 1.** Inactivation Parameters for Enzymes in Carrots and Potatoes<sup>a</sup>

source	enzyme	form	% total activity	$T_{\text{ref}}$ (°C)	$k_{\text{ref}}$ ( $\times 10^3$ ) ( $\text{s}^{-1}$ )	$E_a$ (kJ/mol)	$T$ for $D = 5$ min (°C)
carrot	POD	resistant	50	80	$12.5 \pm 0.17$	$480 \pm 5$	80.3
carrot	PME	labile	80	65	$10.9 \pm 0.75$	$510 \pm 30$	65.7
carrot	PME	resistant	20	70	$11.4 \pm 0.04$	$635 \pm 13$	70.5
carrot	PG	resistant	100	80	$8.7 \pm 0.91$	$411 \pm 29$	81.2
potato	POD	resistant	50	80	$33.6 \pm 3.6$	$478 \pm 35$	83.2
potato	PME	labile	80	65	$56.5 \pm 6.1$	$493 \pm 23$	69.0
potato	PME	resistant	20	70	$7.4 \pm 1.2$	$759 \pm 62$	70.0

<sup>a</sup> Activation energies ( $E_a$ ) and rate constants ( $k_{\text{ref}}$ ) for inactivation at the reference temperature ( $T_{\text{ref}}$ ) were calculated from the Arrhenius plots in Figures 1–4. The temperature required for a  $D$  value of 5 min was calculated from the  $E_a$  and  $k_{\text{ref}}$ . The parameters for POD are for temperatures  $>78$  °C.

different temperature dependences, each described by an  $E_a$  and a  $k_{\text{ref}}$ . With these two values for  $E_a$  and  $k_{\text{ref}}$ , the expected rate of inactivation, the sum of  $k_1$  and  $k_2$ , can be calculated at any temperature by determining the values of  $k_1$  and  $k_2$  according to eq 3. To model our data, values for  $k_1$  were calculated using the  $E_a$  and  $k_{\text{ref}}$  determined from the data at temperatures  $>78$  °C (Table 1). In the low-temperature range, the values for  $E_a$  and  $k_{\text{ref}}$  needed to calculate  $k_2$  could not be determined directly from the experimental data because a limiting slope was not apparent. Instead,  $E_a$  and  $k_{\text{ref}}$  were chosen so that the calculated values for  $k_2$  gave a reasonable fit to the experimental data when plotted as  $\ln(k_1 + k_2)$  versus  $1/T$ . The lines in Figure 1C were obtained when  $E_a$  values of 52 and 55 kJ/mol and  $k_{\text{ref}}$  values ( $T_{\text{ref}} = 60$  °C) of  $9.9 \times 10^{-5}$  and  $6.2 \times 10^{-4} \text{ s}^{-1}$  were assumed for carrots and potatoes, respectively. These activation energies in the low-temperature range are  $1/10$  those determined for inactivation at high temperature, consistent with our assertion that inactivation involves both a high activation energy and a low activation energy process. The reasonable fit between the lines and the data points shows that a two-pathway model can reasonably simulate the experimental data and can be used to predict rates of inactivation over the entire temperature range.

Although the limiting slopes at higher temperatures are the same for both the potato and carrot enzymes and both show similar curvature over the same temperature range, the rates of inactivation for the carrot enzyme are severalfold lower at all temperatures. This could indicate that the carrot enzyme is inherently more thermally stable than the potato enzyme. However, it is also possible that differences in the composition of the solution surrounding the enzyme during the heat treatment affected the kinetics of inactivation. The carrot enzyme was heated in crude carrot juice (pH 6.2), whereas the potato enzyme was desalted and heated in 0.1 M phosphate buffer (pH 6.0). The exact composition and pH of the medium used for heating is known to have a significant effect on the observed inactivation kinetics (1, 28).

Inactivation kinetics have been determined for POD activity with both crude preparations (31–33) and purified enzymes (22, 28, 29) from a number of other sources. In most cases the reported activation energies are substantially lower than what we report here. Although the lower values for the activation energy obtained with purified enzymes could be explained by differences in the environment surrounding the enzyme during heating, this cannot explain the low values reported with other homogenates or intact tissue. It is possible that the peroxidases in carrots and potatoes have unusual thermal stability. However, we have recently determined that the activation energies for inactivation of peroxidases in both tomato and peach homogenates are similar to what we found in carrots and potatoes (manuscript in preparation). The relatively low values for  $E_a$  determined for peroxidases purified from sources such as horseradish and Brussels sprouts may not be typical. It is also

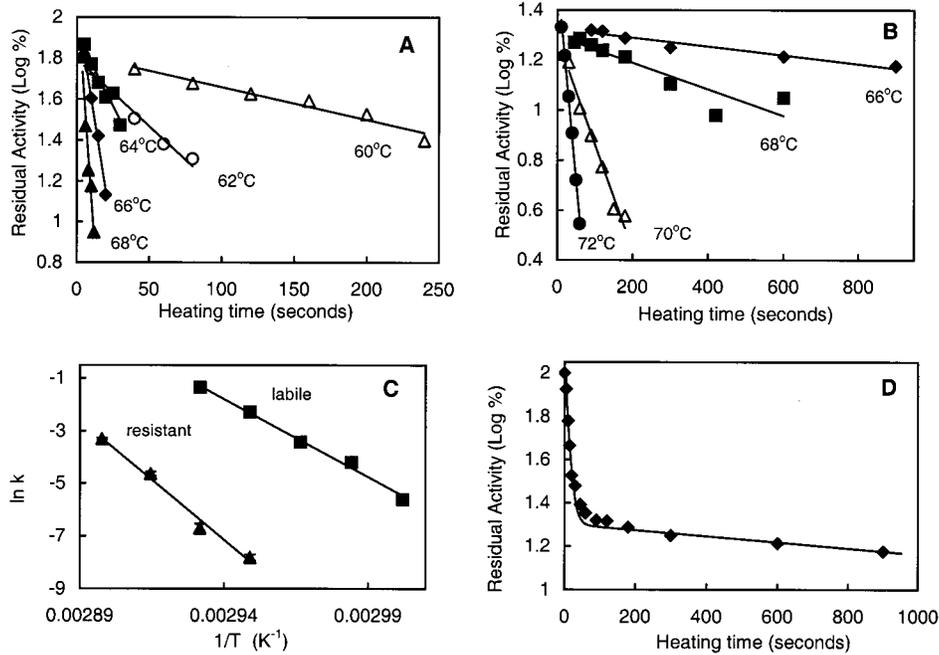
possible that some of the lower estimates result from a failure to note nonlinearities in the Arrhenius plot along with the inclusion of data from a wide range of temperatures. Given the widespread use of POD as an indicator enzyme, further characterization of the inactivation kinetics under a variety of conditions and in a number of commodities may be in order.

**Pectin Methylesterase.** Like POD, PME inactivation kinetics showed strong similarities between carrots and potatoes. In potatoes inactivation was biphasic, indicating the presence of labile and resistant forms. Labile and resistant forms of PME have been shown to occur in a number of other fruits and vegetables including oranges (2), grapefruits (34), tomatoes (35), and green beans (36). Inactivation kinetics for the labile and resistant forms are shown in parts A and B, respectively, of Figure 2. By extrapolating the lines in Figure 2B back to zero it could be estimated that the resistant form constituted  $\sim 20\%$  of the total activity. The Arrhenius plots for these enzymes showed no obvious deviation from linearity (Figure 2C).

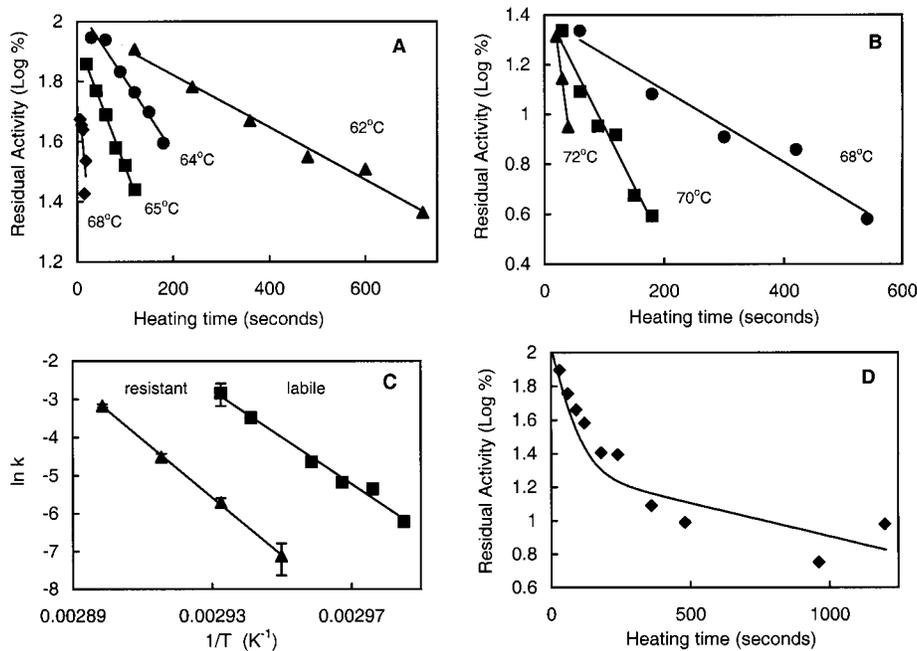
Carrots gave very similar results (Figure 3A–C). Again, labile and resistant forms were apparent. By extrapolating the lines in Figure 3B back to zero, it was again estimated that the resistant form constituted  $\sim 20\%$  of the total activity. Similarly, simple linear Arrhenius plots were obtained for the two forms. Inactivation parameters are summarized in Table 1. From the activation energy and the rate of inactivation at a reference temperature the expected rates of inactivation for each of the two forms of the enzyme can be calculated at any given temperature. From these calculated rates and the assumption that 80% of the activity is labile and 20% is resistant, the expected total level of residual activity versus heating time can be determined. The expected levels of residual activity were calculated for 66 °C (solid lines in Figures 2D and 3D) and agree reasonably well with experimental data for this temperature.

**Polygalacturonase.** Carrots have been shown to contain an exo-polygalacturonase activity (13). The inactivation kinetics of this enzyme are given in Figure 4. This enzyme shows simple first-order inactivation kinetics (Figure 4A) with no evidence of multiple isoforms. The Arrhenius plot for these data is also a simple linear fit with a slope equivalent to an activation energy of 411 kJ/mol. Although this activation energy is similar to those obtained with PME and POD, the range of temperatures required for inactivation was higher, with significant inactivation occurring only at temperatures  $>75$  °C. The temperature required for a  $D$  value of 5 min was 81 °C, higher than that for any of the other carrot enzymes (Table 1).

Our inactivation data do not indicate the presence of multiple isoforms of PG in carrots. It has been reported (13) that two forms of PG can be found in carrot roots. The two forms were distinguished, however, solely on the basis of the salt concentration needed to solubilize them. The minimal data on thermal stability that these authors present indicates very little difference



**Figure 2.** Heat inactivation of pectin methyltransferase in potatoes: (A) heat inactivation of the labile fraction; (B) heat inactivation of the resistant fraction; (C) Arrhenius plot of the inactivation rates from (A) and (B); (D) inactivation rate at 66 °C. The solid line was generated by assuming 80% of the total activity was labile and 20% resistant with inactivation rate constants at 66 °C calculated from the parameters given in Table 1.



**Figure 3.** Heat inactivation of pectin methyltransferase in carrots: (A) heat inactivation of the labile fraction (B) heat inactivation of the resistant fraction; (C) Arrhenius plot of the inactivation rates from (A) and (B); (D) inactivation rate at 66 °C. The solid line was generated by assuming 80% of the total activity was labile and 20% resistant with inactivation rate constants at 66 °C calculated from the parameters given in Table 1.

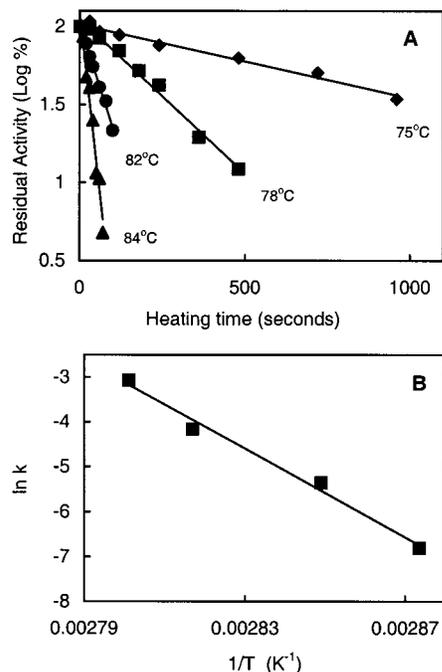
between the two forms. Because we extracted the carrot homogenates with high salt prior to assay, we presumably have both forms in our assay. The simple kinetics that we obtained indicate that the soluble and bound forms have the same thermal stability.

The physiological role of PG in root tissue such as carrots and potatoes is not known. It is also not established what, if any, effects it may have on quality in intact carrots or carrot juice. The fact that it is an exo-PG argues that it may have little role in altering the viscosity of carrot juices and purees. On the other hand, the apparent high heat stability of this enzyme

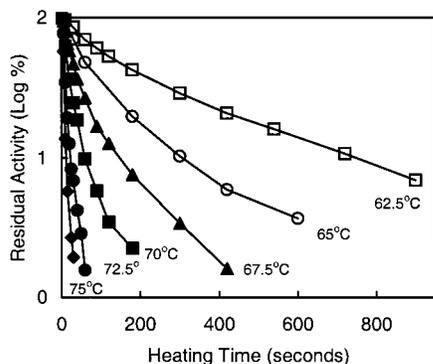
suggests that residual activity may survive some heat treatments. This suggests that a further examination of this enzyme in relation to quality changes may be warranted.

Potatoes have also been shown to contain PG activity (37), and we were able to detect the presence of this activity in a crude potato homogenate. However, the total level of activity was very low; consequently, the accurate determination of inactivation kinetic parameters for this enzyme in a crude preparation was not possible.

**Lipoxygenase and Polyphenol Oxidase.** Two additional quality-related enzymes, LOX and PPO, were investigated in



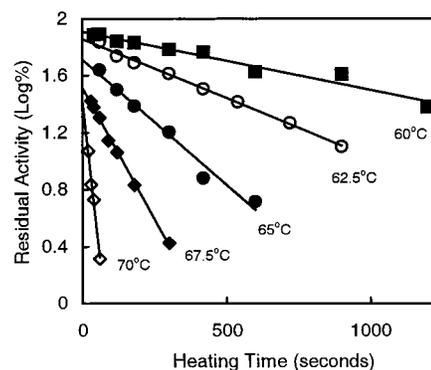
**Figure 4.** Heat inactivation of polygalacturonase in carrots: (A) residual activity versus heating time; (B) Arrhenius plot of the inactivation rates in (A).



**Figure 5.** Heat inactivation of polyphenol oxidase in potatoes.

potatoes. These two enzymes were not investigated in carrots because carrots do not contain detectable levels of LOX (31, 38) and PPO is found only in the peel of carrots (10), which is generally discarded during commercial processing. In potatoes PPO inactivation did not conform to simple first-order kinetics. The plot of the log of residual activity versus heating time (Figure 5) was curved but could not easily be resolved into labile and resistant phases. A more complicated model assuming either multiple isoforms or complex inactivation kinetics would be needed to fit these data.

The inactivation kinetics for LOX were also complex. Straight lines can be reasonably fitted to the inactivation data at each temperature, but the lines do not extrapolate back to a common point (Figure 6). This is inconsistent with a simple first-order model that assumes two isozymes of different thermal stabilities (20). These data could fit such a model only if the relative amounts of the two isozymes are assumed to be different at the different inactivation temperatures. As with PPO, a more complicated model assuming either more than two isozymes or complex inactivation kinetics would be needed to fit these data. Similar results have been obtained for LOX inactivation in a green bean homogenate (24).



**Figure 6.** Heat inactivation of lipoxygenase in potatoes.

It has been reported that potatoes contain two forms of LOX, which can be separated on DEAE-cellulose (11). Reported inactivation kinetics performed on these two forms showed that they differed in thermal stability. This difference was, however, relatively small such that the rate of inactivation of the labile form was only about twice that of the resistant form. This small difference in relative stability would not give rise to the strong deviation from simple first-order kinetics that we observe here.

Because the inactivation kinetics for both PPO and LOX are complex, it is not possible to determine either the inactivation rate at a reference temperature or the activation energies for inactivation. A direct comparison with these parameters determined for inactivation of potato POD and PME is thus not possible. However, it is apparent from an examination of the extent of inactivation resulting from a given time and temperature treatment that LOX and PPO are not as thermally stable as POD or PME. For example, by inspection of Figures 5 and 6 the temperature required to achieve a *D* value of 5 min is ~66 °C for both enzymes. This is lower than the temperatures required to obtain the same *D* value with any of the other potato enzymes (Table 1).

**Conclusions.** Although there were a number of similarities in the thermal inactivation kinetics of carrot and potato enzymes, the two commodities differed in terms of which particular enzymes were the most heat resistant. Both carrots and potatoes have a resistant isoform of POD with an activation energy of ~480 kJ/mol; however, the rates of thermal inactivation of POD in carrot were severalfold lower at all temperatures. Carrot PG had an *E<sub>a</sub>* of 411 kJ/mol, which was similar to the values for POD and PME, but inactivation did not occur at temperatures <75 °C; hence, the *D* value for carrot PG was higher than that for the other enzymes. POD is commonly used by the freezing industry as an indicator of the adequacy of blanching. However, its relationship to quality changes during storage of either raw or processed products is questionable. Other preservation technologies rarely determine the activity of residual enzymes, much less correlate that activity to quality deterioration. Greater understanding of the relationship of specific endogenous enzymes to fruit and vegetable quality and control of residual levels during processing and storage will improve the color, texture, flavor, and nutritional value of processed products.

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