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Effect of Mild-Heat and High-Pressure Processing on Banana Pectin Methylesterase: A Kinetic Study

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Pectin methylesterase (PME) was extracted from bananas and purified by affinity chromatography. The thermal−high-pressure inactivation (at moderate temperature, 30−76 °C, in combination with high pressure, 0.1−900 MPa) of PME was investigated in a model system at pH 7.0. Under these conditions, the stable fraction was not inactivated and isobaric−isothermal inactivation followed a fractional-conversion model. At lower pressure (≤300−400 MPa) and higher temperature (≥64 °C), an antagonistic effect of pressure and heat was observed. Third-degree polynomial models (derived from the thermodynamic model) were successfully used to describe the heat−pressure dependence of the inactivation rate constants.

KEYWORDS: Banana; pectin methylesterase; high pressure; inactivation; kinetics; thermodynamic model

INTRODUCTION

Among nonthermal processing technologies, high-pressure processing is being applied on an industrial scale for some food products marketed in Japan, the United States, and some European countries (*1*). High-pressure processing, in contrast to high-temperature processing, shows a higher specificity toward maintaining the fresh quality of foods because it slightly affects covalent bonds (in the pressure range used). Hence, high-pressure processing at room temperature has very little detrimental effect on food quality attributes such as vitamins, pigments, and flavors (*2*−*6*) and can supply consumers with fresher and higher quality safe food products.

The enzyme pectin methylesterase (pectinesterase, PME, PE, EC 3.1.1.11), which has been found in plants as well as in pathogenic fungi and bacteria, catalyzes the hydrolysis of the methyl ester groups from pectin and leads to the formation of a calcium pectate gel (*7*−*10*). Consequently, its activation, on the one hand, causes cloud loss of juices and nectars (*11*−*14*) and, on the other hand, (i) enhances the texture of fruit and vegetable products (*7*, *15*, *16*), (ii) effectively increases the extracting yield of juices by conventional methods (*17*), and (iii) promotes water removal from the tissues on drying (*18*).

Plant PMEs have been isolated, purified, and studied in terms of pressure−thermal processing stability. In this context, commercial tomato PME was found to be activated under lower pressure (<300 MPa) treatment at mild temperature (60−65 °C) (*19*). Similarly, purified strawberry PME showed extreme pressure stability toward combined pressure−temperature treatment up to 1000 MPa at 10 °C (*20*). For orange PME, a pressure−temperature kinetic diagram for inactivation was published showing an antagonistic effect of pressure and temperature at pressures below 300 MPa and temperatures above 60 °C (*21*). Thus, high-pressure treatment can activate or inactivate plant PMEs, depending on the pressure−temperature level applied. Both effects (activation and inactivation) on PME can be beneficial in the processing of fruit- and vegetable-based products. Pressure−temperature processing stability data for plant PMEs, therefore, are of interest to the food industry.

In the present work, a detailed kinetic study was performed using banana PME. The processing stability of purified banana PME in a model system (i.e., in 20 mM Tris-HCl buffer, pH 7.0) was investigated using combined heat−pressure treatments. This fruit was selected because high-pressure processing might be of interest for the processing of banana juice and nectar.

MATERIALS AND METHODS

Materials. A stock of 15 kg of bananas (Bonita banana: cv. Cavendish, Ecuador) was purchased from a supermarket. Apple pectin (degree of esterification = 70−75%) was obtained from Fluka Chemical Co. (Buchs, Switzerland). All other chemicals were of analytical grade.

PME was extracted from bananas and purified by affinity chromatography on a CNBr-Sepharose 4B−PME inhibitor column (*22*). The purified banana PME obtained was desalted and dissolved in 20 mM Tris-HCl buffer (pH 7.0) (specific activity is 480 units/mg of protein), quickly frozen using liquid nitrogen, and stored at −80 °C for further use.

PME Assay. PME activity was measured by continuous recording of the titration of carboxyl groups released from a pectin solution using

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Table 1. Fitted Rate Constants (min^{-1}) of Fractional-Conversion Inactivation of Purified Banana PME (in 20 mM Tris-HCl buffer, pH 7.0) at Different Combinations of Temperature and Pressure

P (MPa)	30 °C	40 °C	50 °C	55 °C	60 °C	64 °C	67 °C	70 °C	73 °C	76 °C
0.1	ND[a]	ND	ND	ND	ND	0.0060 ± 0.0013[b]	0.0192 ± 0.0028	0.0550 ± 0.0062	0.1950 ± 0.0150	0.6300 ± 0.0220
100	ND	ND	ND	ND	ND	ND	ND	0.0058 ± 0.0011	ND	ND
200	ND	ND	ND	ND	ND	ND	ND	ND	0.0048 ± 0.0001	0.0093 ± 0.0003
300	ND	ND	ND	ND	ND	ND	ND	ND	0.0027 ± 0.0006	0.0055 ± 0.0001
400	ND	ND	ND	ND	ND	ND	ND	ND	0.0034 ± 0.0004	0.0075 ± 0.0002
500	ND	ND	ND	ND	ND	ND	ND	0.0038 ± 0.0009	0.0070 ± 0.0002	0.0116 ± 0.0018
600	ND	ND	ND	ND	ND	ND	ND	0.0093 ± 0.0001	0.0121 ± 0.0005	0.0197 ± 0.0012
700	ND	0.0067 ± 0.0004	ND	ND	0.0119 ± 0.0004	0.0167 ± 0.0006	ND	0.0306 ± 0.0010	0.0347 ± 0.0013	ND
800	0.0319 ± 0.0016	0.0247 ± 0.0016	0.0357 ± 0.0014	0.0354 ± 0.0017	0.0557 ± 0.0026	0.0849 ± 0.0044	ND	0.1387 ± 0.0065	0.1308 ± 0.0006	0.2348 ± 0.0050
850	0.0632 ± 0.0028	0.0796 ± 0.0049	0.0717 ± 0.0048	0.0876 ± 0.0042	0.1166 ± 0.0042	ND	ND	ND	ND	ND
900	0.1217 ± 0.0079	0.1500 ± 0.0091	0.1400 ± 0.0140	0.1410 ± 0.0120	0.1980 ± 0.0100	ND	ND	ND	ND	ND

[a] Not determined. [b] Standard error of regression.

Table 2. Estimated Model Parameters for Purified Banana PME Inactivation Based on the Classical Model (Equation 4), the Third-Degree Thermodynamic Model (Equation 6), and the Third-Degree Polynomial Model (Equation 7) at a Reference Pressure of 850 MPa and a Reference Temperature of 333.15 K (60 °C)

	parameter	classical thermodynamic model (*A*) (eq 4)	third-degree thermodynamic model (*B*) (eq 6)	parameter	third-degree polynomia model (*C*) (eq 7)	units
1	k_0	0.1136 ± 0.0167[a]	0.1100 ± 0.0051	*A*	–2.2184 ± 0.0456	min^{-1}
2	$\Delta V_0^{\ddagger}$	–55.81 ± 3.66	–35.97 ± 2.03	*B*	0.0130 ± 0.0007	$cm^3\ mol^{-1}$
3	$\Delta S_0^{\ddagger}$	188.20 ± 39.12	142.00 ± 14.70	*C*	0.0512 ±0.0052	$J\ mol^{-1}\ K^{-1}$
4	$\Delta\kappa^{\ddagger}$	–0.1110 ± 0.0084	0.0714 ± 0.0146	*D*	–10.0E–6 ± 2.5E–6	$cm^6\ J^{-1}\ mol^{-1}$
5	$\Delta C_P^{\ddagger}$	3368.3 ± 1076.7	2290.4 ± 375.5	*E*	0.00119 ± 0.00020	$J\ mol^{-1}\ K^{-1}$
6	$\Delta\zeta_1^{\ddagger}$	0.1237 ± 0.0767	0.1277 ± 0.0658	*F*	–0.00013 ± 0.00005	$cm^3\ mol^{-1}\ K^{-1}$
7	$\Delta\kappa_2^{\ddagger}$	-	–15.0E–5 ± 1.3E–5	*G*	–25.3E–9 ± 2.3E–9	$cm^6\ J^{-1}\ mol^{-1}$
8	$\Delta\zeta_{2A}^{\ddagger}$	-	0.00031 ± 0.00012	*I*	18.98E–8 ± 8.11E–8	$cm^3\ mol^{-1}\ K^{-1}$
9	$\Delta\zeta_{2B}^{\ddagger}$	-	–0.00579 ± 0.00250	*J*	–3.48E–6 ± 1.77E–6	$cm^3\ mol^{-1}\ K^{-1}$
10	corrected r^2	0.981	0.998	corrected r^2	0.999	
11	SD	0.518	0.154	SD	0.151	

[a] Standard error of regression.

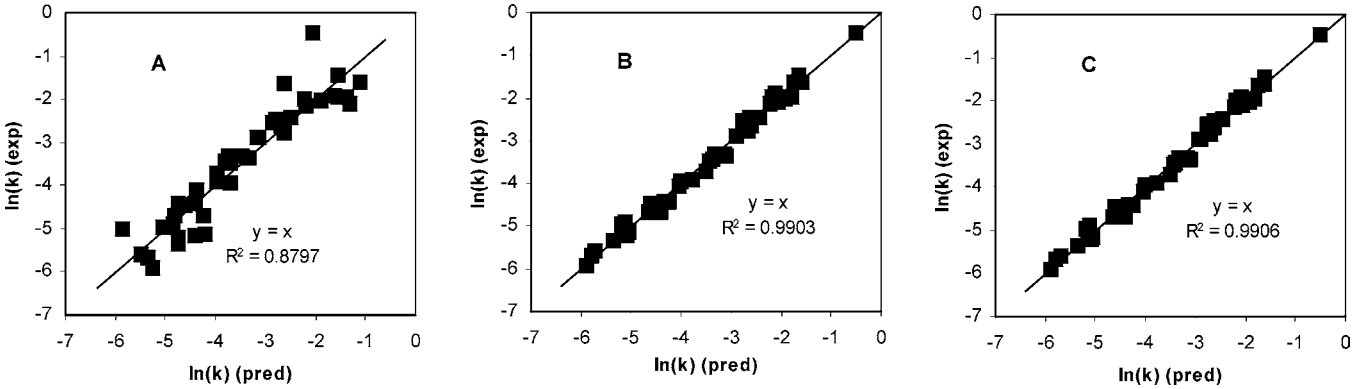

Figure 1. Correlation between the natural logarithm of the experimental *k* values of the isobaric–isothermal inactivation of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) and the natural logarithm of the predicted *k* values according to (**A**) the classical thermodynamic model (eq 4), (**B**) the third-degree thermodynamic model (eq 6), and (**C**) the third-degree polynomial model (eq 7).

an automatic pH-stat (Metrohm, Herisau, Switzerland) and 0.01 N NaOH. Routine assays were performed with a 3.5 mg mL^{-1} apple pectin solution (30 mL) containing 0.117 M NaCl (pH 7.0) at 22.5 °C. The activity unit of PME is defined as the amount of enzyme required to release 1 μmol of carboxyl groups per minute, under the aforementioned assay conditions (*23*).

Heat Inactivation of Purified Banana PME. Isothermal treatments were performed in a temperature-controlled water bath using 200-μL capillaries (Blaubrand, Wertheim, Germany) to enclose the enzyme solution. After treatment, the samples were immediately cooled in ice water. Residual activities of PME were measured within 60 min of storage in ice water. Previous experiments showed the absence of reactivation during this time period.

Combined Heat—Pressure Inactivation of Purified Banana PME. All combined heat−pressure experiments were conducted in a multi-vessel, high-pressure apparatus (eight vessels of 8 mL) (Resato, Roden, The Netherlands), which allows pressurization up to 1000 MPa in combination with temperatures ranging from −20 to 100 °C. The pressure medium is a glycol−oil mixture (TR 15, Resato). Enzyme samples in 0.3-mL flexible microtubes (Elkay, Leuven, Belgium) were enclosed in the pressure vessels, already equilibrated at the inactivation temperature. Pressure was built slowly using a standard pressurization rate of ~100 MPa/min to minimize the temperature rise due to adiabatic heating (*24*, *25*). After pressure buildup, an equilibration period of 2 min to allow the temperature of the pressure medium to evolve to its preset value (input value) was taken into account (*21*). After 2 min of equilibration, one pressure vessel was decompressed and the activity of the corresponding enzyme sample was considered as the blank (A_0). The other seven vessels, each containing one enzyme sample, were then decompressed as a function of time. After pressure release, samples

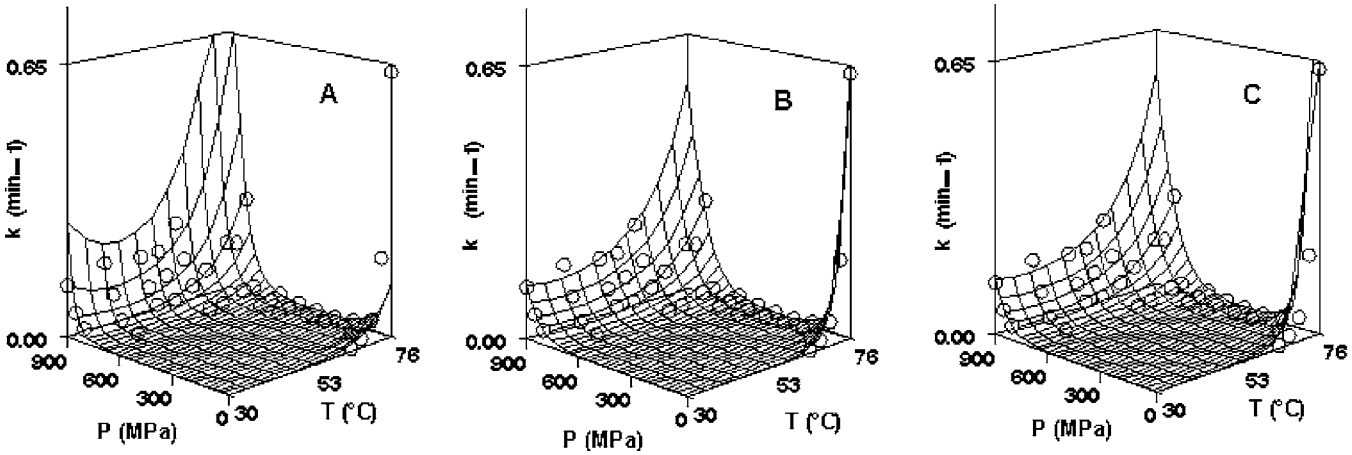

Figure 2. 3D plots for heat–pressure inactivation of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) based on (**A**) the classical thermodynamic model (eq 4), (**B**) the third-degree thermodynamic model (eq 6), and (**C**) the third-degree polynomial model (eq 7); (○) raw data points.

were immediately cooled in ice water and the residual PME activity was measured within 60 min of storage time in ice water. The experiments were performed at combined pressures and temperatures ranging from 100 to 900 MPa and from 30 to 76 °C.

Kinetic Data Analysis. As previously published by Ly Nguyen and co-workers (*22*), the heat−pressure inactivation of purified banana PME followed a fractional-conversion model. This model applies when the enzyme sample contains a stable fraction that is not affected under the processing condition studied (eq 1)

$$A = A_\infty + (A_0 - A_\infty)\exp(-kt) \tag{1}$$

where A_0 and A are, respectively, the initial activity and the remaining activity at time t (min); A_∞ is the remaining activity after prolonged treatment (mL of 0.01 N NaOH/min); and k is the inactivation rate constant (min^{-1}).

Many mathematical models have been formulated that allow the description of temperature−pressure dependence of the inactivation rate constant over a broad range of pressures and temperatures. The most useful thermodynamic-based kinetic model governing the behavior of a system during pressure and temperature change (eq 2) was used as a general equation to describe the heat−pressure inactivation of purified banana PME (*26*−*28*). This model has been successfully applied as a generic model for a number of enzyme inactivation data and can currently be regarded as the most generic model in this field (*29*).

$$\ln(k) = \ln(k_0) - \frac{\Delta V_0^{\ddagger}}{R_T T}(P - P_0) + \frac{\Delta S_0^{\ddagger}}{R_T T}(T - T_0) - \frac{\Delta \kappa^{\ddagger}}{2R_T T}(P - P_0)^2 + \frac{\Delta C_P^{\ddagger}}{R_T T}\left\{T\left[\ln\left(\frac{T}{T_0}\right) - 1\right] + T_0\right\} - \frac{2\Delta \zeta^{\ddagger}}{R_T T}(P - P_0)(T - T_0) \tag{2}$$

In eq 2, P is pressure (MPa); T is absolute temperature (K); P_0 and T_0 are reference pressure (MPa) and absolute temperature (K), respectively; ΔV_0 and ΔS_0 are volume change ($cm^3\ mol^{-1}$) and entropy change (J $mol^{-1}\ K^{-1}$) between native and denatured states, respectively; $\Delta\kappa$ is compressibility factor ($cm^6\ J^{-1}\ mol^{-1}$); ΔC_P is heat capacity (J $mol^{-1}\ K^{-1}$); $\Delta\zeta$ is thermal expansibility factor ($cm^3\ mol^{-1}\ K^{-1}$); k is inactivation rate constant (min^{-1}); k_0 is inactivation rate constant at P_0 and T_0 (min^{-1}); and R_T is the universal gas constant (8.314 J $mol^{-1}\ K^{-1}$).

Recently, Smeller (*30*) suggested a possible modification that in the vicinity of the reference point, one can use the following second-order approximation:

$$T\left(\ln\left(\frac{T}{T_0}\right) - 1\right) + T_0 = \frac{(T - T_0)^2}{2T_0} \tag{3}$$

This approach results in a second-degree polynomial (elliptic) equation:

$$\ln(k) = \ln(k_0) - \frac{\Delta V_0^{\ddagger}}{R_T T}(P - P_0) + \frac{\Delta S_0^{\ddagger}}{R_T T}(T - T_0) - \frac{\Delta \kappa^{\ddagger}}{2R_T T}(P - P_0)^2 + \frac{\Delta C_P^{\ddagger}}{R_T T}\frac{(T - T_0)^2}{2T_0} - \frac{2\Delta \zeta^{\ddagger}}{R_T T}(P - P_0)(T - T_0) \tag{4}$$

When $\Delta\kappa$, ΔC_P, and $\Delta\zeta$ are temperature and/or pressure dependent (*31*, *32*), an extended analysis of the free energy change $\Delta G(T,P)$ (in their cases) or $\ln(k)(T,P)$ (in the present case) is necessary, where higher order terms are also involved.

$$\ln(k) = \ln(k_0) - \frac{\Delta V_0^{\ddagger}}{R_T T}(P - P_0) + \frac{\Delta S_0^{\ddagger}}{R_T T}(T - T_0) - \frac{\Delta \kappa^{\ddagger}}{2R_T T}(P - P_0)^2 + \frac{\Delta C_P^{\ddagger}}{R_T T}\frac{(T - T_0)^2}{2T_0} - \frac{2\Delta \zeta^{\ddagger}}{R_T T}(P - P_0)(T - T_0) + \text{higher-order terms} \tag{5}$$

In general, there are four third-degree terms (*33*):

$$\frac{\Delta \kappa_2^{\ddagger}}{2RT}(P - P_0)^3;\ \frac{\Delta C_{P2}^{\ddagger}}{2RTT_0}(T - T_0)^3;\ \frac{2\Delta \zeta_{2A}^{\ddagger}}{RT}(P - P_0)^2(T - T_0);\ \text{and}\ \frac{2\Delta \zeta_{2B}^{\ddagger}}{RT}(P - P_0)(T - T_0)^2$$

Note that the subscript "2" refers to the coefficients of the higher order terms; however, in the case of pressure−temperature inactivation of banana PME, the term $\Delta C_{P2}^{\ddagger}/2RTT_0\ (T - T_0)^3$ is redundant as indicated by the large standard error (100%). As a consequence, this term was omitted and a reduced version of eq 5 was used (i.e., eq 6).

$$\ln(k) = \ln(k_0) - \frac{\Delta V_0^{\ddagger}}{R_T T}(P - P_0) + \frac{\Delta S_0^{\ddagger}}{R_T T}(T - T_0) - \frac{\Delta \kappa^{\ddagger}}{2R_T T}(P - P_0)^2 + \frac{\Delta C_P^{\ddagger}}{R_T T}\frac{(T - T_0)^2}{2T_0} - \frac{2\Delta \zeta^{\ddagger}}{R_T T}(P - P_0)(T - T_0) + \frac{\Delta \kappa_2^{\ddagger}}{2RT}(P - P_0)^3 + \frac{2\Delta \zeta_{2A}^{\ddagger}}{RT}(P - P_0)^2(T - T_0) + \frac{2\Delta \zeta_{2B}^{\ddagger}}{RT}(P - P_0)(T - T_0)^2 \tag{6}$$

Equation 6 can be rewritten as an empirical polynomial equation as its parameters have no longer a physical meaning:

$$\Rightarrow \ln(k) = A + B(P - P_0) + C(T - T_0) + D(P - P_0)^2 + E(T - T_0)^2 + F(P - P_0)(T - T_0) + G(P - P_0)^3 + I(P - P_0)^2(T - T_0) + J(P - P_0)(T - T_o)^2 \tag{7}$$

where A, B, C, D, E, F, G, I, and J are unknown parameters.

As measures for the quality of model fitting, the corrected r^2 and the model standard deviation (SD) were calculated using eqs 8 and 9, respectively

$$\text{corrected } r^2 = \left[1 - \frac{(m-1)\left(1 - \frac{\text{SSQ}_{\text{regression}}}{\text{SSQ}_{\text{total}}}\right)}{(m-j)}\right] \quad (8)$$

$$\text{SD} = \sqrt{\frac{\text{SSQ}_{\text{residual}}}{(m-j)}} \quad (9)$$

where m is number of observations, j is number of model parameters, SSQ is sum of squares, and SD is standard deviation.

RESULTS AND DISCUSSION

Heat—Pressure Inactivation Kinetics of Purified Banana PME. Heat−pressure inactivation experiments of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) were conducted for different combinations of moderate temperature (30−76 °C) and pressure (0.1−900 MPa). Isothermal−isobaric inactivation of purified banana PME followed a fractional-conversion model (eq 1), and the inactivation rate constants and their standard errors are presented in **Table 1**. From this table, it is clear that there is an antagonistic effect of pressure and temperature in the "low"-pressure ($P \leq 300-400$ MPa)−high-temperature domain (≥64 °C, in the present study). In this range, a pressure increase resulted in a decrease of the observed inactivation rate constant. An antagonistic effect of pressure and temperature is frequently encountered for enzyme inactivation/protein denaturation. This effect is mostly limited to pressures below 300 MPa (*3*, *21*, *28*, *34*−*36*). Pressure stabilization of enzymes/proteins against thermal inactivation/denaturation might be due to counteracting effects of pressure and temperature on the formation or disruption of intramolecular interactions and/or their opposing effects on interactions between enzyme/protein and solvent (water).

Discussing the interaction of enzyme/protein functional groups and solvent (water), Gross and Jaenicke (*37*), Mozhaev and co-workers (*35*), and Barbosa-Cánovas and co-workers (*38*) stated that in the initial step of thermal inactivation, a protein loses a number of essential water molecules, and this loss may give rise to structural rearrangements. High pressure may hamper this process owing to its favorable effect on hydration of both charged and nonpolar groups (*37*, *39*).

Opposing effects of pressure and temperature with respect to hydrophobic interactions and hydrogen bonds have furthermore been put forward as possible explanations for pressure stabilization of enzymes/proteins against thermal inactivation/denaturation. Endothermic hydrophobic interactions are known to be enhanced at elevated temperatures, being maximal at ∼60−70 °C and thereafter decreasing because of a gradual breakdown of the water structure (*40*). Pressure, on the other hand, greatly weakens hydrophobic interactions (*2*, *34*). As to hydrogen bridges, it is generally accepted that these interactions are destabilized by elevated temperature. Pressure, on the other hand, often stabilizes hydrogen bridges (*2*, *3*, *40*).

Modeling of Combined Heat—Pressure Dependence of Inactivation Rate Constants. By fitting eqs 4, 6, and 7 on the experimental data, we estimated the model parameters using nonlinear regression analysis (Proc NLIN, SAS) (**Table 2**).

For the three model versions, no tendency was found by plotting residuals (differences between experimental and predicted k values, respectively) as a function of temperature, pressure, experimental k value, and predicted k value (data not shown). In addition, parity plots of the natural logarithm of the predicted k values based on eqs 4, 6, and 7 versus the natural logarithm of the experimental k values, respectively, were established (**Figure 1**). The deviation from the bisector can be considered as an indicator for the inaccuracy of the models. The less the experimental and predicted k values mutually differ, the more successful the model is. Good agreements between

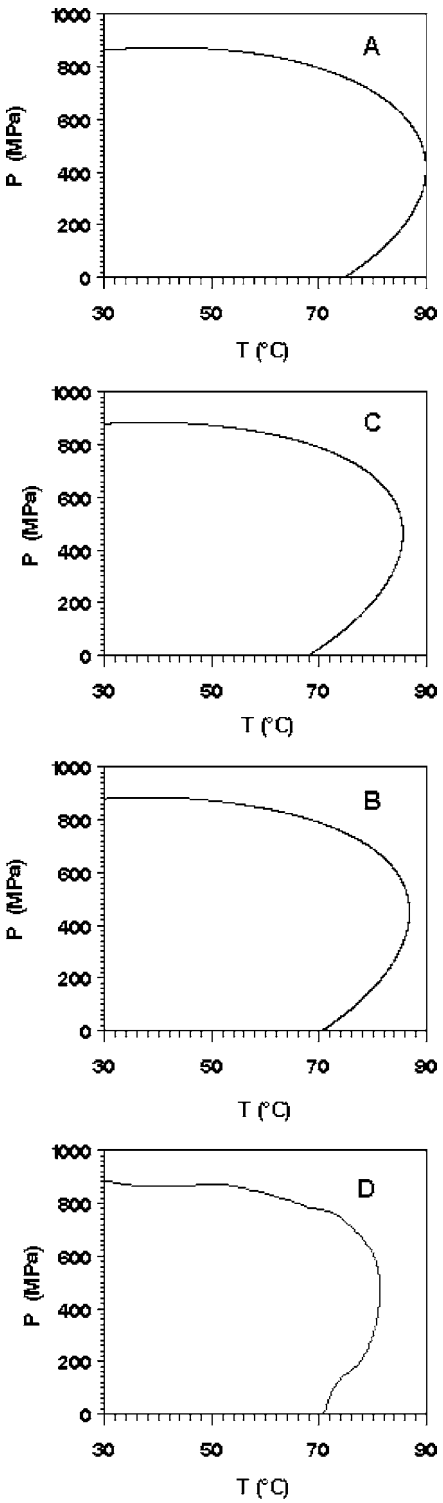

Figure 3. Heat–pressure isorate contour plots of 95% inactivation of purified banana PME (in 20 mM Tris-HCl buffer, pH 7.0) for a total process time of 30 min ($k = 0.099858$ min^{-1}) based on (**A**) the classical thermodynamic model (eq 4), (**B**) the third-degree thermodynamic model (eq 6), (**C**) the third-degree polynomial model (eq 7), and (D) raw data.

the natural logarithm of the predicted k values and that of the experimental k values were observed for the three model versions. It is obvious that the third-degree models better fit the experimental data [$R^2 = 0.9903$ (eq 6) and 0.9906 (eq 7)] as compared to the "classical" model [$R^2 = 0.8797$ (eq 4)]. Graphically, the third-degree models show better fitting in the areas of low temperature−high pressure and high temperature−low pressure (**Figure 2**). A similar conclusion was drawn for the heat−pressure inactivation of purified carrot PME (*33*). In addition, a comparison of correlation coefficients and residuals based on eqs 6 and 7 allowed us to conclude that the third-degree polynomial model with unknown parameters (eq 7) can also be used to adequately describe the heat−pressure dependence of banana PME. However, because there is not a big difference between the quality of the third-degree thermodynamic and that of the third-degree polynomial fittings, the thermodynamic model should be favored, because this is the theoretically correct one. In the case of the polynomial model, the $1/RT$ factor is built into the constants of the polynomial and, therefore, the change of the temperature is not taken into account exactly.

By inserting all model parameters of **Table 2** into eqs 4, 6, and 7, respectively, pressure−temperature combinations resulting in specific preset inactivation rate constants for purified banana PME were simulated and depicted in isorate contour plots (**Figure 3**). Smeller and Heremans in 1997 (*32*) discussed that as one applies the thermodynamic model with only first- and second-order terms the shape of the contour plot will be elliptic or hyperbolic. To leave out the higher order terms is equivalent to the assumption that $\Delta\kappa$, ΔC_P, and $\Delta\xi$ are independent of temperature and pressure. If any of these shows temperature or pressure dependence, higher order terms appear not to be negligible. In cases when higher order terms become important and are included in the model, the contour plot will be a distorted ellipse.

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