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Modelling of High Hydrostatic Pressure inactivation of pectinmethylesterase from persimmon fruit

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Abstract

Pectin methylesterase (PME) can be found in significant levels of activity in various fruits and vegetables such as persimmon. The cloud loss of juices is mainly attributed to the action of PME. High Hydrostatic pressure (HHP) inactivates PME while maintaining the quality characteristics of the fruit juices, resulting in products of superior quality. Considerable research has been reported on PME inactivation by HHP. The objective of this work was to study and model the effect of HHP processing on the inactivation of PME obtained from fresh persimmon *(Diospyros virginiana)* pulp. Amulti-parameter model that estimates the inactivation rate constant of Persimmon Pme at a wide variety of pressures (500-800MPa) and temperatures (40-70ºC) was developed, enabling the proper design for PME inactivation.

Keywords: Persimmon, pectinmethylesterase, high hydrostatic pressure, inactivation, model

Introduction

Pectinolytic enzymes such as pectinmethylesterase (PME) can be found as endogenous enzymes in several sources (fruits and vegetables) among which orange, tomato, peach and persimmon. Cloud loss accompanied by gelation of juice concentrates is a major problem associated with juice quality deterioration, which has been attributed primarily to the activity of PME, a cell-wall bound pectic enzyme released into the juice during extraction. PME hydrolyses the C_6 methyl esters of galacturonic acid residues in pectins, gradually decreasing their degree of esterification. Demethylated pectin interacts with calcium ions causing precipitation of insoluble calcium pectate, as well as other cloud forming compounds entrapped (Goodner et al., 1999). The action of PME affects the sensory characteristics of the juice-product. HHP inactivation of PME has been the subject of many studies (Polydera et al., 2004, Van der Broeck et al., 2000, Knorr et al., 1993, Katsaros et al., 2005). Compared to conventional processes, HHP treatment has been found to be less detrimental to low molecular weight food compounds, due to the stability of covalent bonds to high pressure. Therefore, vitamins, pigments, flavouring agents and other compounds associated with sensory, nutritional and health related qualities of the product, are not greatly affected by HHP processing (Hoover et al., 1993). The objectives of this work was to study the HHP and thermal inactivation kinetics of PME obtained from persimmon. The controlled inactivation of this enzyme is necessary for the optimal process design.

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Materials and Methods

PME activity assay

PME activity was measured using a modification of Rouse and Atkins (1955) method. Titration (Autotitrator Titralab TIM 854, Radiometer Analytical, France) with NaOH of the carboxylic groups generated by PME during the hydrolysis of a pectin solution at pH 7.5 and a temperature of 30º C was conducted. Juice sample of 2 ml adjusted to pH 7.5 with NaOH 0.2 N was added to 50 ml 1% apple pectin (70–75% degree of esterification, Fluka Bio-Chemika, Buchs, Switzerland) solution containing 0.3 M NaCl which was also previously adjusted to pH 7.5 with 0.2 N NaOH at 30°C. The consumption of NaOH was recorded during a period of about 20 min. The slope dVNaOH/dt was determined in the linear part of the titration curve. PME activity of juice sample, which is calculated by Eq. (1) and expressed as microequivalents per min is directly proportional to the slope:

$$A\ (\text{units/mL}) = \frac{(\text{mL NaOH})(\text{N NaOH})10^3}{(mL\ \chi\upsilon\mu o\acute{\upsilon})(\text{min})} = \frac{mL\,\text{NaOH}\,0.02\cdot 10^3}{2\text{mL}\cdot(\text{min})} \quad \text{(Equation 1)}$$

Persimmon PME extraction

Persimmon PME was extracted from persimmon pulp according to the following method: equal volumes of persimmon pulp and distilled water containing 0.2M NaCl were mixed. The mixture was homogenised (Bagmixer, Interscience, France) for 15 minutes and then it was stored at 5°C (Sanyo MIR 153, Sanyo electric co., Japan) for 10 minutes. After storage, it was centrifuged at 5000 r/min for 10 minutes. The supernatant after centrifugation was stored into polyethylene bags at –30°C, until used for thermal and HHP treatments.

Thermal treatment

PME obtained from persimmon pulp was thermally inactivated at temperatures in the range of 70-90°C. The experiments were conducted using capillaries for quick temperature transfer. The capillaries were immersed into waterbaths (MEMMERT WB-10). After thermal treatment the capillaries were immediately transferred into iced water until PME activity was measured.

High pressure treatment

Persimmon “extracted juice” was placed into multilayered bags (PP-aluminium leaf-PE) for HHP experiments. HHP inactivation experiments were conducted at various combinations of pressure (500–800 MPa) and temperature (40-70°C) for appropriate time periods. High pressure treatments were achieved using a pilot scale HHP equipment with a maximum operating pressure and temperature of 1000 MPa and 100°C respectively (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Holland) consisting of an operation high pressure unit with a pressure intensifier and a multivessel system consisting of six vessels of 45 mL capacity each. Vessels were surrounded by liquid circulating jackets connected to a heating-cooling system. Pressure was released after preset time intervals independently for each vessel by opening the pressure valve. Pressure and temperature were constantly monitored and recorded (in 1s intervals) during the process.

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Results & Discussion

PME inactivation at ambient pressure

Thermal inactivation of persimmon PME was investigated for temperatures ranging from 70 to 90ºC. As can be seen from Figure 1, thermal inactivation of PME followed first order reaction kinetics (Eq.2),

$$A/A_o=\exp(-kt) \quad \text{(Equation 2)}$$

where A is the PME activity, A_o is the PME activity in zero time, k is the inactivation rate constant and t is time of processing. The inactivation rate constants (k) and the corresponding D-values of thermal inactivation were estimated and they are shown in Table 1. The higher the treatment temperature it was, the higher the inactivation rate constant and the lower the estimated D-value were.

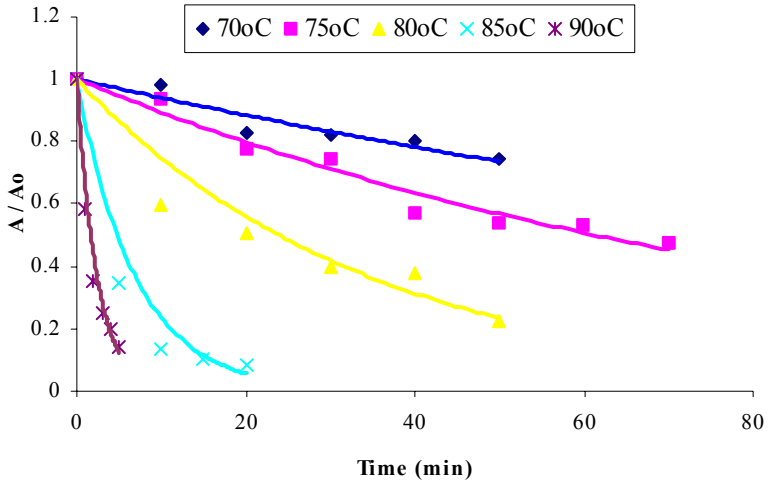

Figure1. Thermal inactivation of Persimmon PME at 70-90ºC

Table1. Inactivation rate constants (min^{-1}) and D- values (min) of thermal inactivation of persimmon PME at 70-90ºC

Temperature (C)	Inactivation rate constant k (1/min)	D-value (min)
70	0.0061	377
75	0.0113	203
80	0.0290	79.5
85	0.1447	15.9
90	0.4188	5.50

The effect of temperature on Persimmon PME inactivation rate was described adequately by Arrhenius equation (Eq. 3), as illustrated in Figure 2,

$$k_T = k_{ref} \cdot \exp\left[-\frac{E_A}{R}\cdot\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \qquad \text{Equation 3}$$

where: k_c: rate of Persimmon PME inactivation at a storage temperature T, k_{ref}: Persimmon PME inactivation rate at a reference temperature $T_{ref\ (80C)}$, Ea: Activation energy (J/mol), R: gas constant (8.314 J/(mol×K)) and temperatures in absolute scale (K). The activation energy of thermal incativation was determined to be 272 kJ/mol (R^2=0.98).

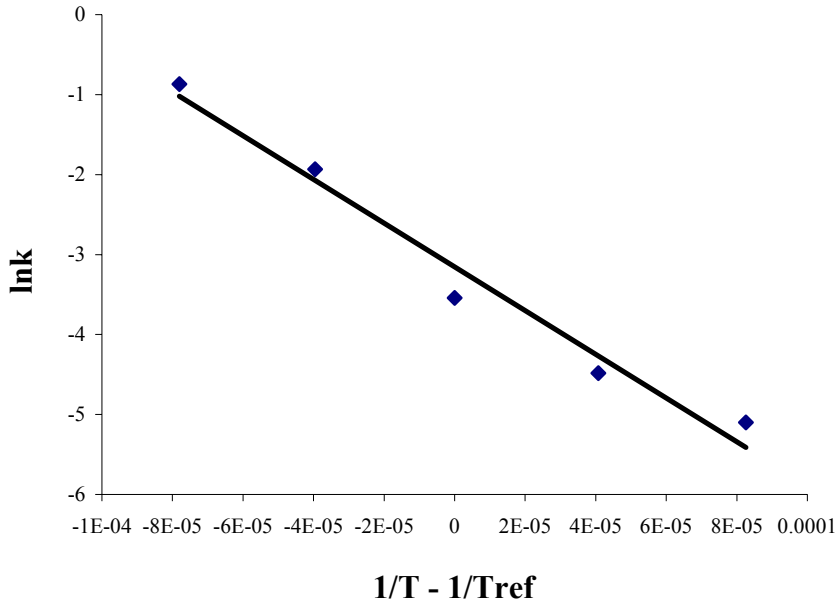

Figure 2. The effect of temperature (70-90ºC)on the thermal inactivation of Persimmon PME

PME inactivation as a function of pressure

Investigation of the effect of pressure and temperature on the inactivation of Persimmon PME was performed. In Fig. 3 the effect of pressure on the inactivation of Persimmon PME at 40°C is shown. As can be seen from Fig. 3, HHP inactivation of Persimmon PME followed first order kinetics, as in thermal inactivation. In Fig. 4, the effect of pressure on the inactivation of the enzyme is shown at a higher process temperature (60°C). The inactivation rate constants were higher compared to the corresponding constants obtained from the 40°C treatment. PME inactivation was faster with increasing processing pressure at all temperature levels tested. A synergistic effect of temperature and pressure was observed at all pressure-temperature conditions studied.

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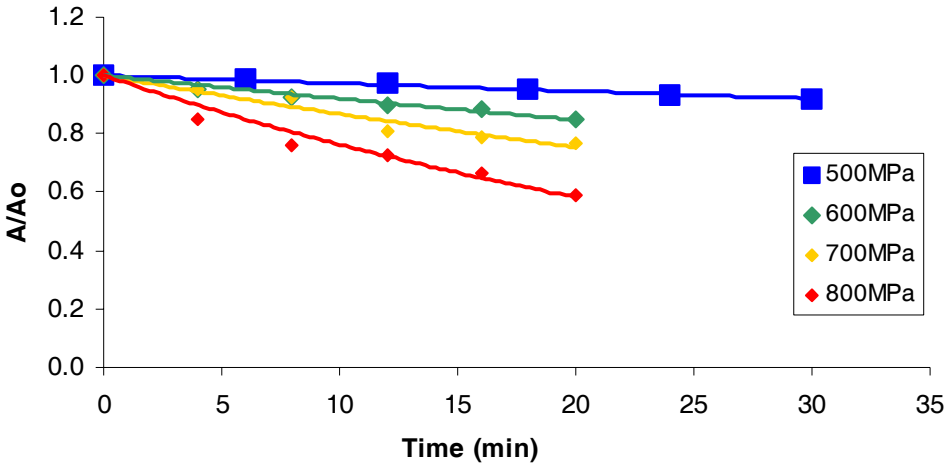

Figure 3. PME inactivation during processing at 40°C and various isobaric conditions (500-800MPa)

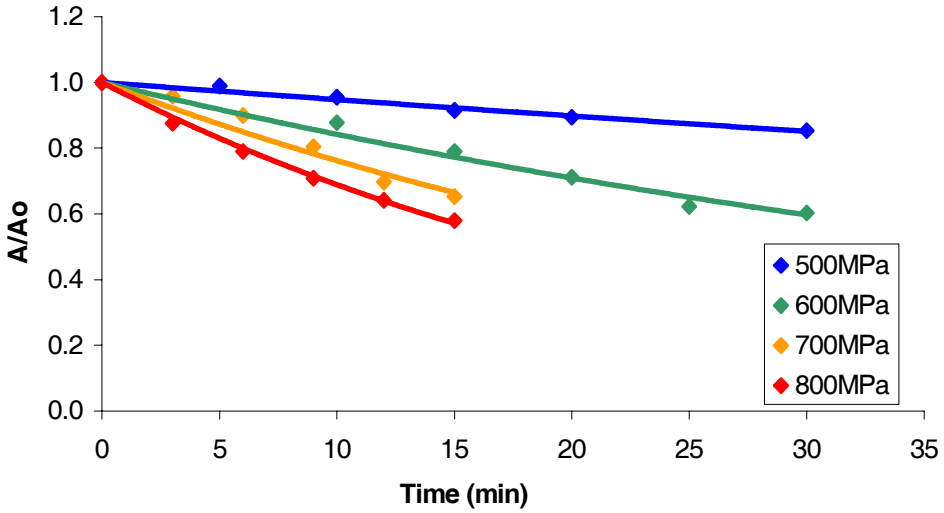

Figure 4. PME inactivation during processing at 60°C and various isobaric conditions (500-800MPa)

The effect of pressure on the inactivation rate constant was expressed through the activation volume Va, as expressed from the equation 4.

$$\ln k_P = \ln k_{P_{ref}} - \frac{V_a}{RT}\left(P - P_{ref}\right) \qquad \text{(Equation 4)}$$

The activation volumes were estimated for all temperatures tested (Table 2). The effect of temperature process on the activation volume was expressed by a linear equation (Fig.5), (Eq.5).

$$V_a = a\left(T - T_{ref}\right) + V_{a,ref} \qquad \text{(Equation 5)}$$

Table 2. The activation volumes at all temperatures tested

Temperature (°C)	V_a (ml/mol)
40	-19.26
50	-17.99
60	-17.44
70	-17.11

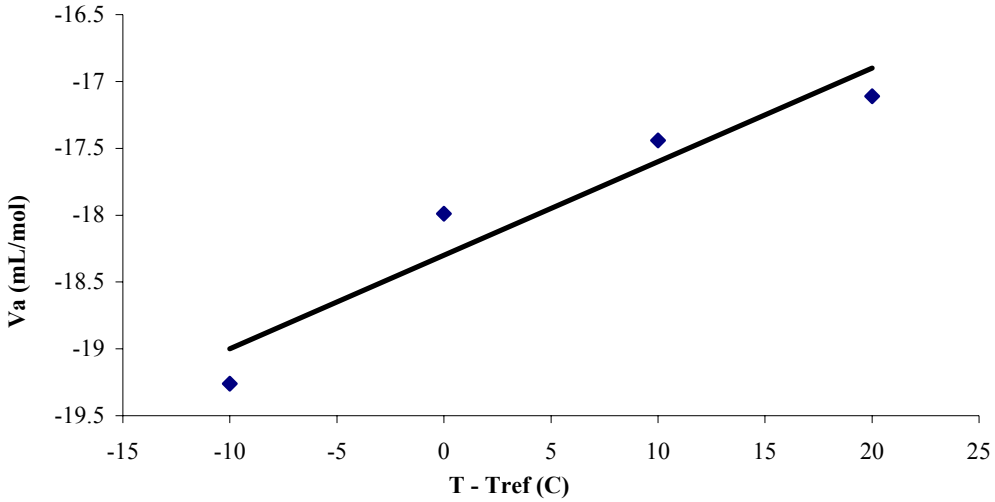

Figure 5. The activation volumes as a function of temperature processing at a T_{ref} of 50ºC

Negative activation volumes indicate that PME inactivation was favoured by pressure. Increase of temperature resulted in reduced absolute values of activation volume, meaning that inactivation rates became less pressure dependent.

PME inactivation as a function of temperature at HHP conditions

The temperature effect on the PME inactivation during isobaric processing at high pressure levels of 500 and 800MPa is shown in Figures 6 and 7 respectively. At all high pressure conditions the inactivation rates increased as temperature increased.

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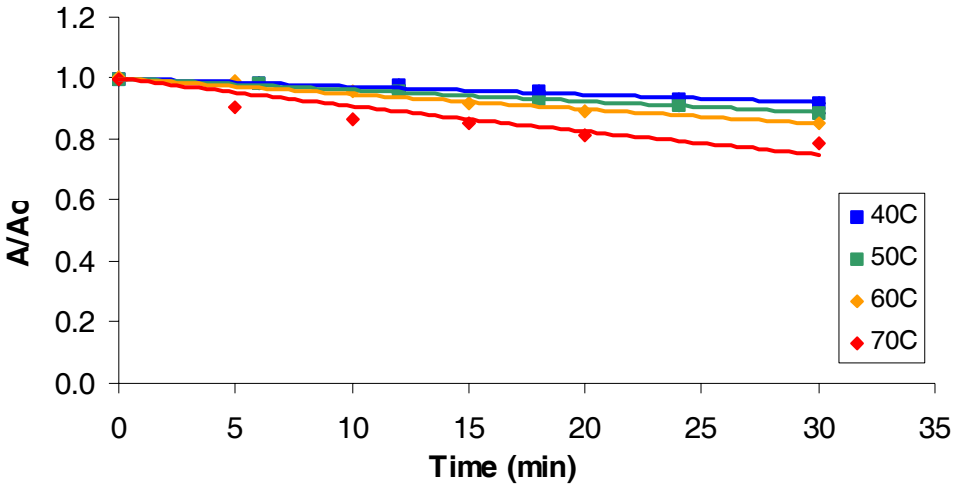

Figure 6. Effect of temperature on the inactivation of Persimmon PME at constant pressure 500MPa

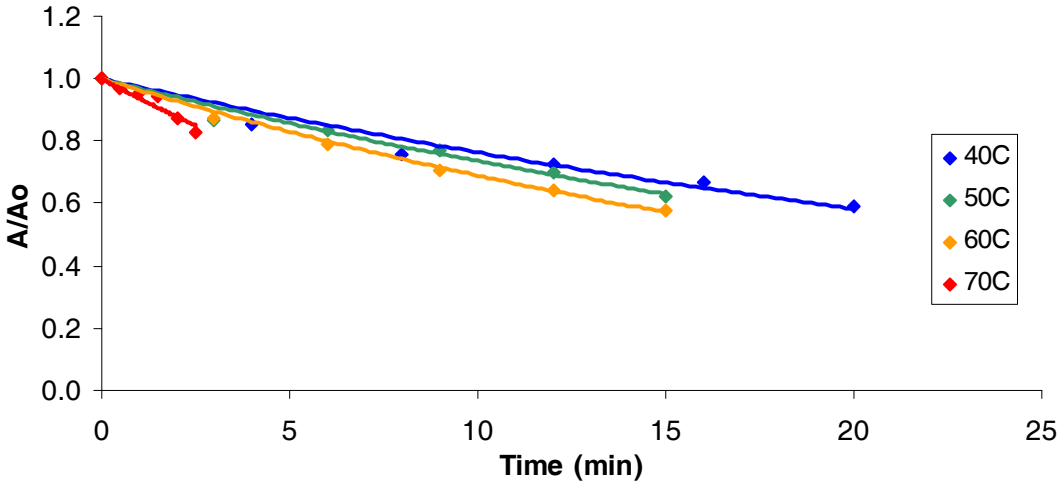

Figure 7. Effect of temperature on the inactivation of Persimmon PME at constant pressure 800MPa

The effect of temperature on the inactivation rate constants was expressed by the Activation energy Ea, estimated from the Arrhenius equation (Eq.6).

$$\ln k = \ln k_{ref,T} - \frac{E_a}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \qquad \text{(Equation 6)}$$

where T_{ref} is the reference temperature (50ºC) and k_{ref} is the activation rate at $T_{ref.}$

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Activation energy values were estimated for all pressures tested (Table 3). The E_a values decreased with increasing pressure indicating less temperature dependence of the enzyme inactivation rate at higher pressures (Fig.8).

Table 3. Activation energy values at all pressures tested

Pressure (MPa)	E_α (kJ/mol)
500	36,29
600	33,56
700	25,97
800	24,87

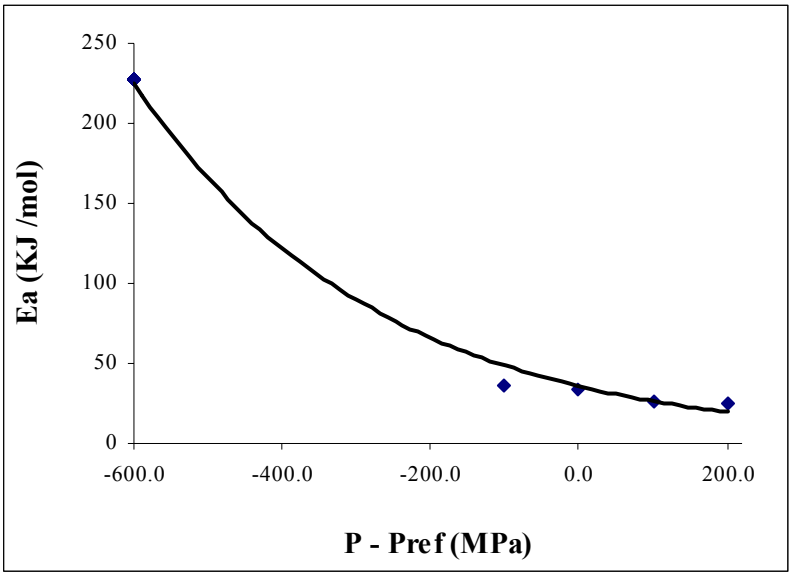

Figure 8. Effect of pressure on the activation energy of Persimmon PME

The effect of the pressure processing on the Activation energy values was expressed by an exponential equation (Eq.7),

$$E_\alpha = E_{\alpha,0} \exp[- b \cdot (P-P_{ref})] \qquad \text{(Equation 7)}$$

where P_{ref} = 600 MPa, E_{ao} = 36 kJ mol^{-1} (activation energy at P_{ref}) and b = 0.0031 MPa^{-1} (R^2=0,98).

PME inactivation as a function of temperature and pressure

The Decimal reduction times of PME obtained from Persimmon were estimated for all pressure-temperature conditions studied (Table 4). The inactivation rate constants were also estimated at all conditions studied and the effect of combined temperature and pressure on the inactivation rate constants is illustrated in Figure 9.

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Table 4. The D-values (min) of persimmon PME inactivation at all pressure-temperature conditions

T(˚C) / P(MPa)	40˚C	50˚C	60˚C	70˚C
500	853	590	426	239
600	281	162	133	85,6
700	163	97,6	84,7	65,2
800	85,3	74,8	61,7	35,2

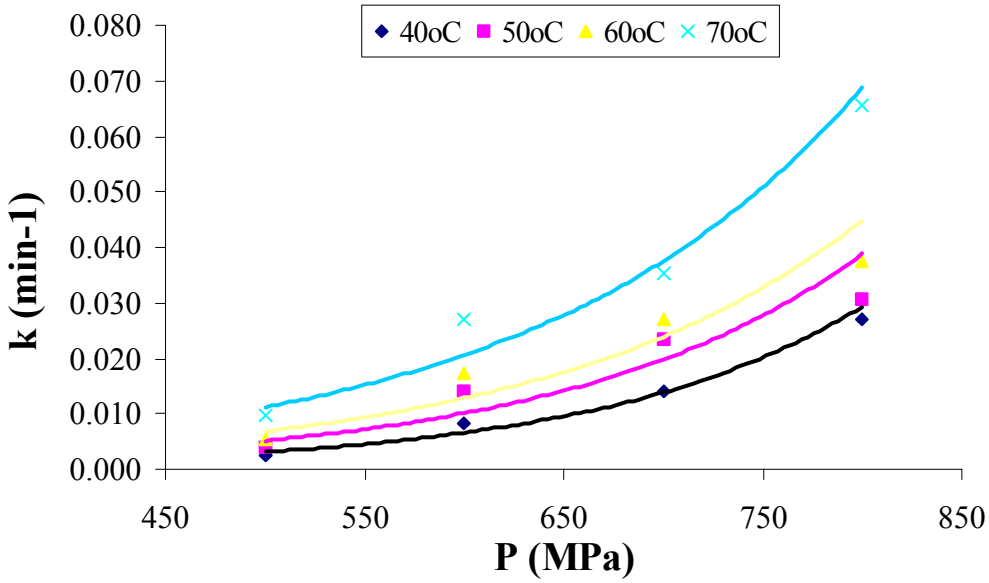

Figure 9. Effect of pressure and temperature on the inactivation rate constants of Persimmon PME

Having as variables pressure and temperature processing and taking also into account the effect of temperature on the activation volume and the effect of pressure on the activation energy, the inactivation rate constant at different temperature and pressure conditions can be expressed by a multi-parameter equation (Eq.8).

$$k = k_{refP,T} \cdot \exp\left\{-\frac{E_{aP}}{R} \cdot \exp\left[-B\left(P - P_{ref}\right)\right] \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}}\right) - \frac{A \cdot \left(T - T_{ref}\right) + V_{aT}}{R} \cdot \frac{\left(P - P_{ref}\right)}{T}\right\}$$

(Equation 7)

The parameters of the Equation 8 were determined through the SYSTAT non-linear regression routine (Table 5). All the values of parameters (A, B, E_a, V_a, k_o) were determined with satisfactory results (R^2, observed vs. Predicted = 0.97) (Table 5). A good correlation between experimental and predicted values of inactivation rate constants was established

(Fig.10). Symbols refer to experimental values, while the lines represent the predicted values from the inactivation model (Eq. 8).

Table 5. Parameters of the model of Persimmon PME inactivation determined through the SYSTAT non-linear regression routine

Parameter	Estimate	Lower (95% CI)	Upper (95% CI)
K_0 (min-1)	0.016	0.013	0.019
E_a (kJ/mol)	29	10.6	47.2
V_a (mL/mol)	-14.3	-17.4	-11.3
A(mL/mol·K)	*-0.004*	*-0.005*	*-0.003*
B(MPa^{-1})	*0.451*	*-0.905*	*1.807*

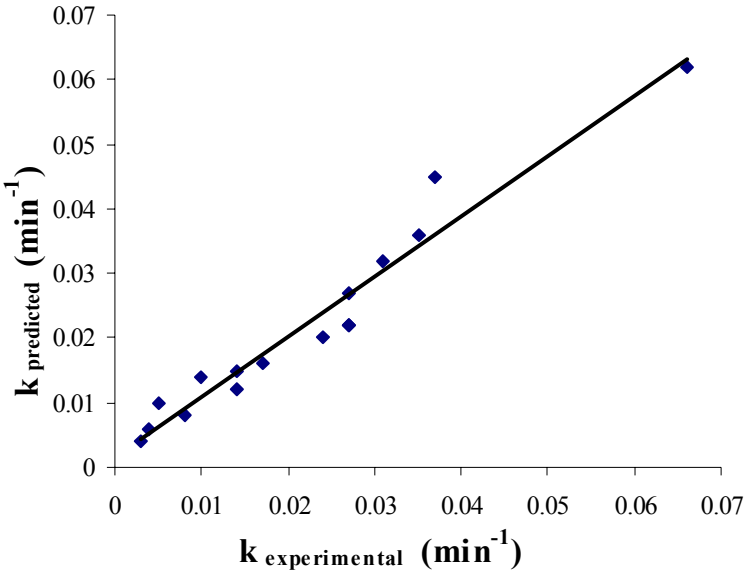

Figure 10. Comparison of experimental inactivation rate constants and predicted from the model

The inactivation rate constant of Persimmon PME can be determined from the model for a wide range of pressures (500-800MPa) and temepratures (40-70ºC). The 3-dimensional Figure 11 illustrates the enzyme inactivation rate as a function of both pressure and temperature processing.

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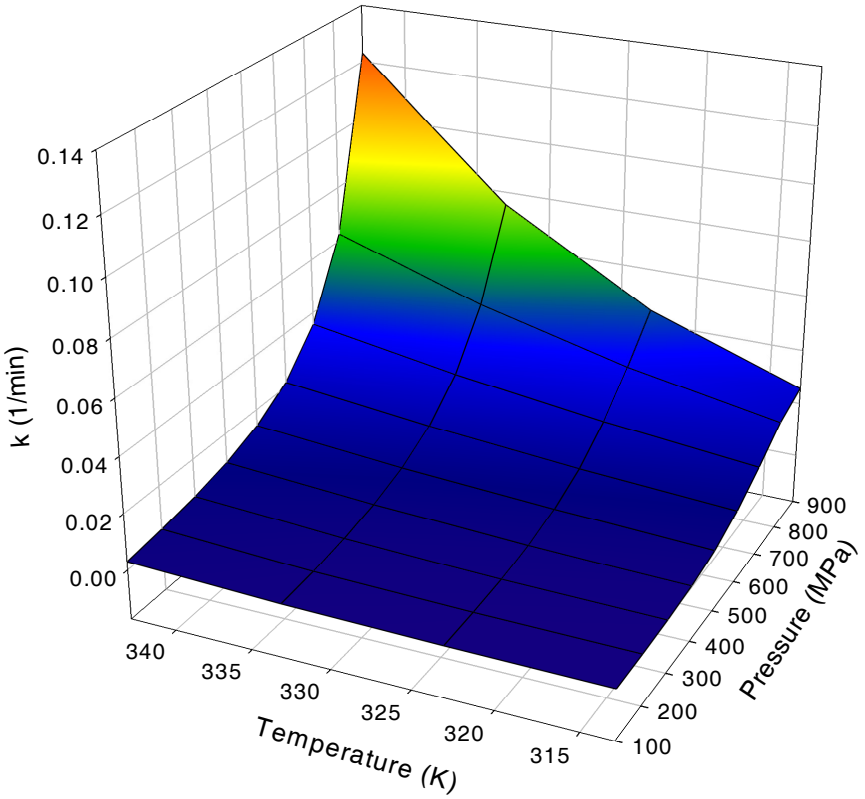

Figure 11. Effect of pressure and temperature on the inactivation rate constants of Persimmon PME

Conclusions

Inactivation of Persimmon PME followed a first order kinetics both in thermal and HHP treatment. The inactivation rate constants of the treated enzyme can be expressed as a function of temperature and pressure enabling a proper design of high pressure treatments. The effect of pressure on the activation volume V_a was determined through a linear function and the effect of temperature on the activation energy (E_a) was determined through an exponential function respectively. Aiming at a better quantitative comparison of different treatments and an optimal process design, a composite mathematical model, which describes the PME inactivation rate as a function of pressure and temperature conditions, taking into account the dependence of both activation energy and activation volume on the applied pressure and temperature respectively, was explored.

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