Effect of Temperature and/or Pressure on Tomato Pectinesterase Activity → purified PME

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The activity of tomato pectinesterase (PE) was studied as a function of pressure (0.1−900 MPa) and temperature (20−75 °C). Tomato PE was rather heat labile at atmospheric pressure (inactivation in the temperature domain 57−65 °C), but it was very pressure resistant. Even at 900 MPa and 60 °C the inactivation was slower as compared to the same treatment at atmospheric pressure. At atmospheric pressure, optimal catalytic activity of PE was found at neutral pH and a temperature of 55 °C. Increasing pressure up to 300 MPa increased the enzyme activity as compared to atmospheric pressure. A maximal enzyme activity was found at 100−200 MPa combined with a temperature of 60−65 °C. The presence of Ca^{2+} ions (60 mM) decreased the enzyme activity at atmospheric pressure in the temperature range 45−60 °C but increased enzyme activity at elevated pressure (up to 300 MPa). Maximal enzyme activity in the presence of Ca^{2+} ions was noted at 200−300 MPa in combination with a temperature of 65−70 °C.

Pressure Temp

Ca^{2+}

Keywords: *Tomato pectinesterase; thermal stability; pressure stability; kinetics; activation*

INTRODUCTION

Pectinesterase (PE) is widely distributed in higher plants and catalyzes the demethylation of pectin, thereby generating acidic pectin with a lower degree of esterification and methanol. The enzymatic reaction is responsible for cloud destabilization of several fruit and vegetable juices (Rombouts et al., 1982; Nath and Ranganna, 1977). This phenomenon is ascribed to an interaction of deesterified pectin and calcium ions forming insoluble pectates that coprecipitate with the pulp particles in the juice (Krop, 1974). On the other hand, partially deesterified pectin can easily be depolymerized by polygalacturonase (PG), an enzyme that is found in large quantities in tomatoes (Pozsar-Hajnal, 1975). The latter step results in a dramatic decrease of consistency of tomato products (Porretta et al., 1995; Thakur et al., 1996, Lopez et al., 1997). To prevent both quality defects and the accompanying decreased marketability of the products, PE should be inactivated.

However, the food industry can also benefit from a controlled activation of PE. Positive effects of this enzymatic reaction have been reported with regard to texture improvements of fruits and vegetables among others. After freezing and canning of vegetables, the texture is often inferior to that of fresh prepared products because the heat treatment necessary to ensure safety produces extensive pectin hydrolysis and loss of firmness (Steinbuch, 1976; Stanley et al., 1995). This quality defect can be overcome by activating PE. Indeed, firming effects have been observed in cauliflower (Hoogzand and Doesburg, 1961), cherries (Taillan et al., 1992; Alonso et al., 1997), tomatoes (Hsu et al., 1965), potatoes (Bartolome and Hoff, 1972; Anderson et al., 1994), carrots (Lee et al., 1979; Stanley et al., 1995), and green beans (Van Buren et al., 1960, 1988; Steinbuch, 1976; Stanley et al., 1995) after blanching of the products at the optimal temperature for PE activity. It is assumed that deesterification of the pectic substances in the cell wall promotes firming, either by reaction of the free carboxyl groups with divalent ions that are present in the tissue or, more directly, by formation of gellike structures of the pectinic acid produced by the enzyme (Bartolome and Hoff, 1972). Besides this, a decreased susceptibility for heat-induced β-degradation of demethylated pectin has been described in the literature (Keijbets, 1974; Fuchigami, 1987; Stanley et al., 1995). However, it should be mentioned that after attaining the desired degree of esterification, PE should be inactivated because a too pronounced deesterification might result in uncookable products (Fuchigami, 1987; Sajjaanantakul et al., 1989).

Activation as well as inactivation of PE can be attained by thermal treatment. However, thermal treatment is often accompanied by sensorial quality damage. Recently, as an answer to an increased demand for minimally processed products that resemble fresh products, high-pressure processing has been introduced in the food industry. This new technology has the potential to inactivate vegetative microorganisms and several food quality related enzymes (e.g., peroxidase, lipase, lipoxygenase, polyphenol oxidase) while maintaining the original food quality characteristics such as flavor, color, vitamins, and nutrients (Hoover et al., 1989; Hayashi, 1992; Hendrickx et al., 1998; Van Loey et al., 1998; Van den Broeck et al., 1998). An additional advantage is provided by a potential activation of enzymes. Indeed, enzymes may display activation or inactivation, depending on the volume changes associated with changes in the tertiary and quaternary structure of the enzyme at

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10.1021/jf990569n CCC: $19.00
Published on Web 02/01/2000

the pressure level applied (Heremans, 1982; Cheftel, 1992).

Thermal inactivation of tomato PE has already been described in the literature (De Sio et al., 1995; Laratta et al., 1995; Castaldo et al., 1996). However, pressure inactivation is less documented and kinetic information, indispensable for the introduction of high-pressure technology in the food industry, is lacking. Also, systematic kinetic studies related to the catalytic activity of tomato PE under combined pressure and/or temperature treatment are not available. Therefore, the objective of this work is to study the effect of pressure and temperature on the activity of tomato PE on a kinetic basis. Moreover, the influence of some intrinsic factors, such as pH and Ca^{2+} ions, on the enzyme activity is evaluated.

MATERIALS AND METHODS

PE and Media. Tomato PE [EC 3.1.1.11, Sigma (Bornem, Belgium), product P-6763] was purchased as a lyophilized powder containing 145 units/mg of solid. One unit will release 1 μequiv of acid from pectin within 1 min at a pH of 7.5 and a temperature of 30 °C (Sigma). Tomato PE dissolved in deionized water was used as a model system. A concentration of 58 units/mL was used for inactivation studies. For activation studies, concentrations ranged from 5.8 to 58 units/mL.

Inactivation Studies. *Activity Assay, Based on the Formation of Galacturonic Acid.* The remaining activity of PE after thermal or combined pressure–temperature treatment was determined titrimetrically. Titration was performed at a pH of 7.0 and a temperature of 22 °C. The reaction mixture in the standard assay method consisted of 250 μL of PE sample and 30 mL of a 0.35% apple pectin solution [70–75% esterification, supplied by Fluka (Bornem, Belgium)] containing 0.125 M NaCl. During hydrolysis at 22 °C, the pH was maintained at 7.0 by the addition of 0.01 N NaOH using an automatic pH-stat titrator (Metrohm, Berchem, Belgium). Every 15 s the consumption of 0.01 N NaOH was recorded during the 10 min reaction period. The PE activity is proportional to the rate of NaOH consumption ($\Delta V_{NaOH}/\Delta t$) (Crelier et al., 1995).

Thermal Treatment. Isothermal inactivation experiments were performed in a water bath with temperature control. To ensure isothermal heating during inactivation, the enzyme solution was enclosed in capillary tubes (Hirschmann, 1.15 mm i.d., 150 mm length). After preset time intervals, the capillaries were withdrawn from the water bath and immediately cooled in ice water. The remaining activity of PE was measured after 10–120 min of storage in ice water. During storage, no reactivation of the enzyme was observed. The temperature range studied varied from 57 to 65 °C.

Combined Pressure–Temperature Treatment. To perform isobaric–isothermal experiments, a laboratory scale, multivessel high-pressure equipment (HPIU-10.000 serial no. 95/1994, Resato, Roden, The Netherlands) was used. The apparatus allows pressurization up to 1000 MPa in combination with temperatures ranging from −20 to 100 °C. High pressure is generated using a pressure intensifier in the central pressure circuit. The pressure medium is a glycol–oil mixture (TR15, Resato). The temperature is controlled by a thermostated mantle, which surrounds each vessel and which is connected to a cryostat. This apparatus is suited for kinetic studies, since eight individual vessels (volume = 8 mL, diameter = 10 mm, length = 100 mm) can be subjected to the same pressure level and the same temperature level.

Isobaric–isothermal experiments were performed as follows: Flexible microtubes (0.3 mL, Elkay, Overijse, Belgium), filled with solution to be pressurized, were enclosed in the pressure vessels already equilibrated at a preset temperature. Pressure was built up slowly (100 MPa/min) to minimize adiabatic heating. After the desired pressure had been reached, the individual vessels were isolated so that the pressure was maintained in the vessels until the valves were opened. On the basis of previous research (Weemaes et al., 1997), an equilibration period of 1–2 min to allow the temperature to evolve to its desired value (input value) was taken into account. By starting the time course of the experiment ("zero point") after this equilibration period, the process could be considered as an isobaric–isothermal treatment. At that moment, 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 after preset time intervals. After pressure release, the samples were immediately cooled in ice water.

After 10–120 min of storage in ice water, the residual activity was measured. During storage no reactivation of the enzyme was observed. The pressure range studied varied from 100 to 900 MPa at a temperature of 40 and 60 °C.

Activation Studies. *Activity Assay, Based on the Formation of Methanol.* The enzyme activity during thermal or combined pressure–temperature treatment was determined by measuring the release of MeOH as a function of time. The enzyme reaction was initiated by adding 250 μL of PE to 30 mL of pectin solution. Apple pectin [70–75% esterification, supplied by Fluka (Bornem, Belgium)] concentration ranged from 0.2 to 0.8% (w/v). The pH during the enzymatic reaction was controlled by dissolving pectin in a McIlvaine buffer at pH 4.0 and a 0.05 M Tris-HCl buffer at pH 7.0, 7.2, 7.5, or 8.0.

The amount of MeOH formed was determined spectrophotometrically according to a method of Klavons and Bennett (1986). According to this method, methanol is oxidized to formaldehyde with alchohol oxidase, followed by condensation with 2,4-pentanedione to obtain 3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine (Wood and Siddiqui, 1971). This colored product is determined spectrophotometrically at 412 nm.

Alcohol oxidase [EC 1.1.3.13, Sigma (Bornem, Belgium), product A-2404] from Pichia Pastoris was purchased as a solution with a specific activity of 25 units/mg of protein. One unit will oxidize 1 μmol of methanol to formaldehyde per minute at pH 7.5 and 25 °C (Sigma).

Thermal Treatment. Isothermal conditions, studied during the enzymatic reaction catalyzed by PE, were approached by preheating pectin solution at the desired temperature. After preheating, the enzymatic reaction was initiated and 1 mL of this pectin–PE solution was pipetted into individual Pyrex tubes and incubated for preset time intervals in a thermostated water bath. After thermal treatment, tubes were withdrawn from the water bath and the reaction was quenched by a heat shock (heating for 2 min at 86 °C). After cooling of the solutions, the amount of MeOH formed was determined spectrophotometrically. For each experiment, a blank was run; that is, the same experiment was performed without the addition of PE. The amounts of methanol formed during the blank were subtracted from the values obtained for enzymatic hydrolysis of pectin. The temperature range studied varied from 20 to 65 °C.

Combined Pressure–Temperature Treatment. To approach isobaric–isothermal conditions, pectin solution was preheated to the desired temperature. After preheating of the substrate, the enzyme reaction was initiated at atmospheric pressure by the addition of PE solution. Flexible microtubes were filled with the pectin–PE solution and pressurized, following the procedure previously described. After pressure treatment, the reaction was quenched by a heat shock. Solutions were cooled, and the amount of methanol formed was determined spectrophotometrically. As for the thermal treatment, for each experiment a blank was taken into account.

The pressure range studied varied from 100 to 300 MPa while temperatures varied from 20 to 75 °C.

Data Analysis. *Inactivation of Tomato PE.* First-order reactions can be described by the following equation:

$$dA/dt = -kA \quad (1)$$

Under isothermal and isothermal–isobaric conditions, the

Table 1. D, z_t, and z_p Values for Thermal and Combined Pressure–Temperature Inactivation of Tomato PE in Water

	D (min)					z_t
	40 °C	57 °C	60 °C	63 °C	65 °C	value (°C)
0.1 MPa	ND[a]	37.40 ± 1.57[b]	15.17 ± 0.42	4.71 ± 0.12	2.20 ± 0.11	6.45 ± 0.29
800 MPa	ND	ND	93.85 ± 5.84	ND	ND	ND
850 MPa	ND	ND	52.25 ± 2.37	ND	ND	ND
900 MPa	48.44 ± 6.19	ND	29.60 ± 1.39	ND	ND	ND
z_p MPa	ND	ND	199.6 ± 1.8	ND	ND	ND

[a] ND, not determined. [b] Standard errors are shown.

inactivation rate constant k can be determined from a plot of $\ln(A_t/A_0)$ versus time (eq 2).

$$\ln(A_t/A_0) = -kt \tag{2}$$

The temperature dependence of k is given by the Arrhenius relationship with an activation energy (E_a); eq 3 gives the linearized form:

$$k = k_{ref} \exp\left[\frac{E_a}{R_t}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \tag{3}$$

The pressure dependence of k is expressed by the activation volume (V_a), as presented in eq 4 (Morild, 1981). This relation

$$k = k_{atm} \exp(-PV_a/R_pT) \tag{4}$$

is valid at a constant temperature. In the area of food processing, it is common to characterize first-order reactions in terms of D and z values (thermal death time concept). The decimal reduction time (D value) is the time, at a given temperature and/or pressure, needed for a 90% reduction of the initial activity. The relation between the D value and the more general inactivation rate constant (k) is given by eq 5.

$$D = \ln(10)/k \tag{5}$$

The temperature dependence of the D value is given by the z_t value. The z_t value equals the temperature increase necessary to obtain a 10-fold decrease of the D value. The z_t value is, in fact, an alternative to the activation energy E_a.

Analogous to the log-linear relationship between D value and temperature, there is often a log-linear relationship between D value and pressure (see eq 6), whereby z_p is defined

$$\log D = \log D_{ref} + \frac{D_{ref} - P}{z_p} \tag{6}$$

as the pressure increase necessary to obtain a 10-fold decrease of the D value.The z_p value can be used as an alternative for the activation volume V_a.

On the basis of experimental data, D values were calculated from the linear regression of the 10-based (log) logarithm of the activity retention versus processing time. The z_t and z_p values were estimated from linear regression of log(D) versus T and log(D) versus P, respectively.

Activation of Tomato PE. The reaction catalyzed by PE was followed by measuring the release of methanol during thermal or combined pressure–temperature treatment. The activity of PE (micrograms of MeOH per milliliter of pectin solution per minute) was estimated from the initial linear part of the curves obtained by plotting the amount of MeOH formed as a function of time. Linear regressions were performed using the SAS package (SAS, 1994).

RESULTS AND DISCUSSION

Inactivation of Tomato PE by Temperature. Isothermal inactivation of tomato PE in deionized water (58 units/mL) was studied in a temperature range from 57 to 65 °C. Inactivation could be accurately described by a first-order kinetic model as can be seen from a plot of log(A_t/A_0) versus time (see Figure 1). The corresponding estimated kinetic parameters are summarized in Table 1. Estimated D values varied between 37 and 2 min, depending on the temperature applied. The temperature sensitivity of the D value, or the z_t value, was calculated as 6.5 °C.

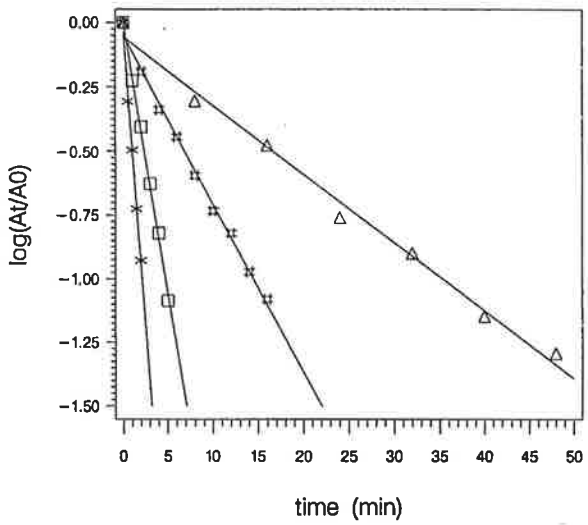

Figure 1. Thermal inactivation of tomato PE at 57 (△), 60 (#), 63 (□), and 65 (*) °C.

Also, De Sio et al. (1995) found that thermal inactivation of tomato PE was exponential. However, they noted a biphasic behavior when plotting D values versus temperature, with z_t values of 11.2 °C when determined within the temperature range 73–78 °C and 27.8 °C when determined within the temperature range 78–88 °C. Crelier et al. (1995) studied the thermal degradation of tomato PE in aqueous solution and in tomato in the temperature range 60–75 °C. According to them, log-scale plots of the residual activity versus time showed two phases, suggesting the coexistence of (at least) two forms of PE, one of them being more thermoresistant. Depending on the isoenzyme, they estimated z_t values of 9.8 and 6.2 °C, respectively. Laratta et al. (1995) isolated three isoforms of tomato PE and characterized each form by heat stability. All three forms revealed a heat inactivation kinetic that displayed an exponential behavior in the temperature range between 70 and 90 °C. Depending on the isoenzyme, they reported z_t values of 23, 15, and 24 °C.

Inactivation of Tomato PE by Combined Pressure–Temperature Treatment. The influence of pressure on thermal inactivation of tomato PE (58 units/mL of deionized water) was investigated at two different temperatures: a temperature of 60 °C, whereby tomato PE inactivates at atmospheric pressure, and a temperature of 40 °C, whereby no thermal inactivation of tomato PE was observed. The pressure range studied

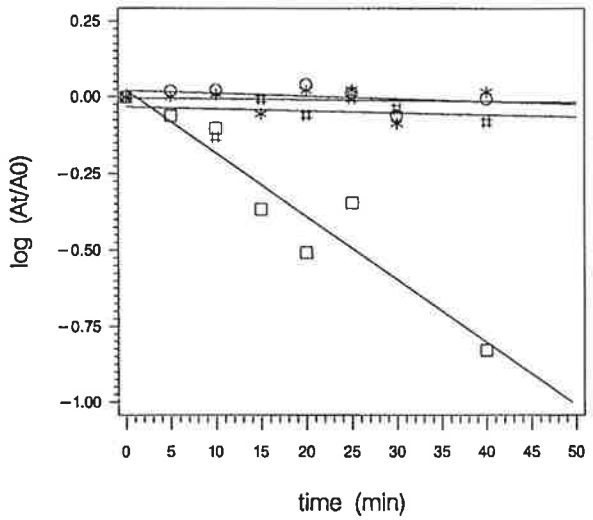

Figure 2. Pressure–temperature inactivation of tomato PE at 40 °C in combination with pressures of 100 MPa (*), 300 MPa (○), 700 MPa (#), and 900 MPa (□).

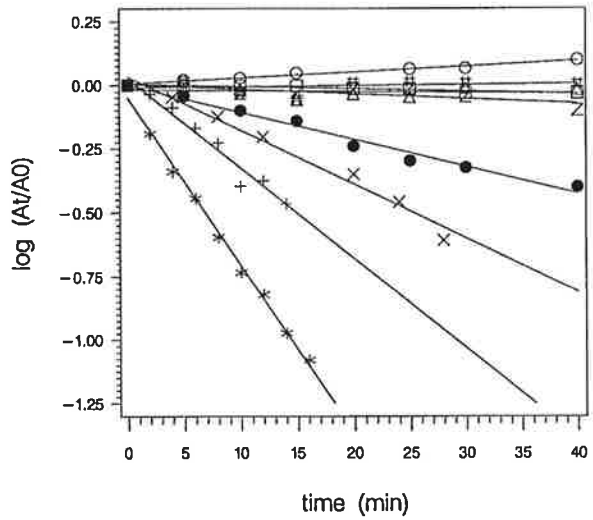

Figure 3. Pressure–temperature inactivation of tomato PE at 60 °C in combination with pressures of 100 MPa (○), 200 MPa (#), 300 MPa (□), 500 MPa (△), 700 MPa (Z), 800 MPa (●), 850 MPa (×), 900 MPa (+), and atmospheric pressure (*).

varied from 100 to 900 MPa. After combined pressure–temperature treatment, the residual activity was measured.

In Figures 2 and 3, the logarithm of the remaining activity is plotted versus time for pressure treatment at, respectively, 40 and 60 °C. In the case of denaturation, a coagulation of the enzyme was observed after combined pressure–temperature treatment. This behavior was not found for thermal inactivation. Suzuki and Taniguchi (1972) also reported that proteins denatured by pressure coagulate more easily in comparison with those denatured by heat. As for thermal treatment, a first-order inactivation was observed. By following the remaining activity of tomato PE as function of storage time, it was confirmed that the denatured enzyme did not reactivate after pressure treatment.

At 40 °C, no inactivation of the enzyme was observed for pressures up to 700 MPa (see Figure 2). A further increase in pressure inactivated tomato PE, which points to a small synergistic effect of pressure and temperature at 40 °C. At 900 MPa a D_{40} value of 48 min was estimated.

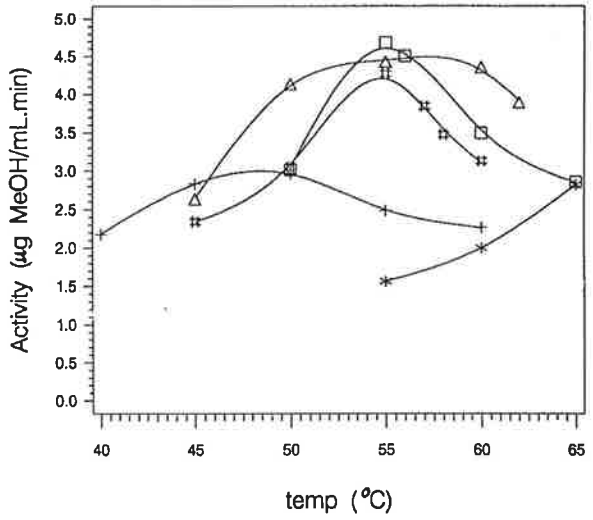

Figure 4. Enzyme activity at atmospheric pressure as function of temperature at pH 4.0 (*), pH 7.0 (△), pH 7.2 (□), pH 7.5 (#), and pH 8.0 (+).

At 60 °C, on the other hand, an antagonistic effect of pressure and temperature was observed; that is, thermal inactivation of tomato PE was strongly counteracted by pressures in the range 100–500 MPa. The antagonistic effect is illustrated in Figure 3. Increasing pressure from atmospheric pressure to 500 MPa suppressed inactivation. A further increase in pressure to 850 MPa slightly increased the inactivation (see Table 1), but even at 900 MPa and 60 °C ($D_{60\,°C,900MPa}$ =30 min) the inactivation was still slower as compared to the same treatment at atmospheric pressure ($D_{60\,°C}$ = 15 min). It can thus be concluded that, although tomato PE is rather heat labile, it is very pressure resistant. Crelier et al. (1995) also mentioned the antagonistic effect of pressure and temperature on the inactivation of tomato PE.

It is also worthwhile mentioning that after a treatment of tomato PE at 60 °C and 100 MPa, and measurement of the residual activity, a small increase in the activity of the enzyme was observed (activity of 126% after 40 min at 60 °C and 100 MPa compared to the untreated enzyme). It might be that the enzyme is activated by a limited conformational change that occurred during pressure–temperature treatment. An increased activity after pressure treatment is also reported for other enzymes. Asaka and Hayashi (1991), Asaka et al. (1994), and Butz et al. (1994) noticed an increased activity of polyphenol oxidase after pressure treatment at 400–500 MPa and room temperature. Likewise, pressurization caused a remarkable activation of peroxidase after treatments carried out at 300–500 MPa (Anese et al., 1995). Also, Cano et al. (1997) reported an activation of polyphenol oxidase, peroxidase, and pectinesterase for treatments carried out in the 250–400 MPa range and at room temperature.

Activation of Tomato PE by Temperature at Atmospheric Pressure. The enzymatic reaction catalyzed by tomato PE was studied as a function of temperature and pH (pH 4.0–8.0). Results are visualized in Figure 4. The estimated activity of tomato PE is summarized in Table 2.

In a preliminary test, experiments were performed with different concentrations of enzyme and substrate to identify an appropriate experimental setup. The enzyme concentration was adjusted so that the amount

Table 2. Activity of Tomato PE at Atmospheric Pressure as a Function of Temperature, pH, and Ca^{2+} Ions

	activity of tomato PE (μg of MeOH/mL of pectin solution, min)					
temp (℃)	pH 4.0	pH 7.0	pH 7.2	pH 7.2 and Ca^{2+}	pH 7.5	pH 8.0
40	ND[a]	ND	ND	ND	ND	2.18 ± 0.17[b]
45	ND	2.64 ± 0.19	ND	ND	2.34 ± 0.04	2.83 ± 0.07
50	ND	4.13 ± 0.19	3.01 ± 0.06	2.59 ± 0.62	3.05 ± 0.50	2.96 ± 0.32
55	1.56 ± 0.06	4.43 ± 0.24	4.69 ± 0.19	3.30 ± 0.16	4.27 ± 0.05	2.48 ± 0.08
56	ND	ND	4.51 ± 0.30	ND	ND	ND
57	ND	ND	ND	ND	3.84 ± 0.48	ND
58	ND	ND	ND	ND	3.47 ± 0.41	ND
60	1.99 ± 0.12	4.36 ± 0.12*	3.49 ± 0.10	3.01 ± 0.08	3.13 ± 0.56	2.26 ± 0.12
62	ND	3.89 ± 0.09*	ND	ND	ND	ND
65	2.82 ± 0.15	ND	2.86 ± 0.15	2.59 ± 0.21	ND	ND

[a] ND, not determined. [b] Standard errors are shown.

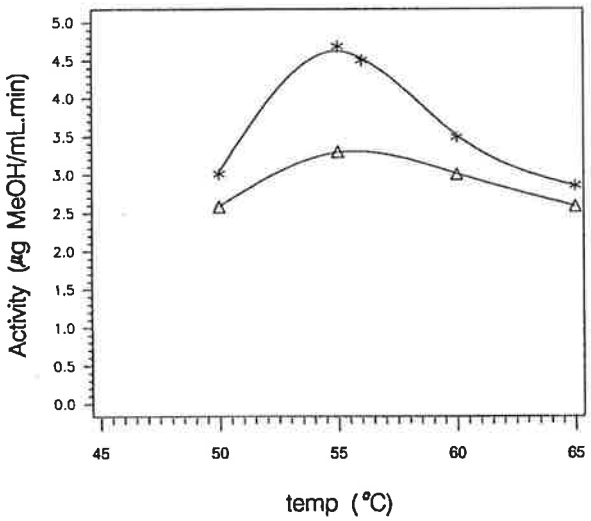

Figure 5. Enzyme activity at atmospheric pressure as function of temperature at pH 7.2 in the absence (*) and presence (△) of 60 mM Ca^{2+}.

of methanol formed by PE increased linearly with time during at least 7 min. In this way, the activity of PE could be accurately estimated from the slope of methanol formed versus time. The pectin concentration was increased gradually until substrate saturation was attained. For all experiments described below, an enzyme concentration of 14.5 units/mL and a substrate concentration of 0.4% was used. Under these conditions, PE saturation by the substrate ([S] > K_m) was maintained, and hence maximal enzyme activity could be determined.

At neutral pH, PE was clearly more active and an optimal temperature was found at 55 ℃. At pH 7.0, a broader temperature optimum was observed as compared to pH 7.2 and 7.5. Both at lower and higher pH values, the activity reduced, which is a confirmation of previous results (Termote et al., 1977; Versteeg et al., 1978), and the temperature optimum shifted to higher and lower temperatures, respectively.

At a pH of 7.2, the effect of Ca^{2+} ions (60 mM) on the activity of tomato PE was investigated. Results are visualized in Figure 5, and the estimated activities are presented in Table 2. As for the reaction in the absence of Ca^{2+} ions, the enzyme activity appeared to increase with increasing temperature, reached a maximum at 55 ℃, and declined after 60 ℃. No shift in temperature optimum was observed. Figure 5 also illustrates that the enzyme activity clearly reduced in the presence of 60 mM Ca^{2+} ions. This is in contradiction to a study of Alonso et al. (1997). They found that PE was optimally active at $CaCl_2$ concentrations between 60 and 70 mM. At concentrations >80 mM, an inhibition of the enzyme reaction was observed. Inhibition of the enzymatic reaction by Ca^{2+} ions can be explained by a competitive displacement for PE binding sites on pectin (Snir et al., 1995). Carboxylate groups located in the vicinity of the $-COOCH_3$ groups that are subjected to hydrolysis by PE normally interact with the active site of the enzyme (Rexova-Benkova and Markovic, 1976). In the presence of high concentrations of salt, metal ions bind to these groups and therefore inhibit the enzyme activity (Nari et al., 1991). The competition of orange PE binding sites on pectin by calcium is also illustrated by Charnay et al. (1992) using Lineweaver–Burk plots. Low concentrations of ions, on the other hand, can activate the enzyme. This effect is also ascribed to an interaction of the ions with the substrate rather than with the enzyme. Blocks of carboxylate groups may trap enzyme molecules, preventing them from reacting with the $-COOCH_3$ groups to be hydrolyzed (Nari et al., 1991). Metal ions decrease this inhibition by binding to carboxylate groups of the pectin. Warrilow and Jones (1995) stated that different forms of PE in tomato might show differing responses to salt concentration.

Activation of Tomato PE by Temperature and Elevated Pressure. The effect of increasing pressure on PE-catalyzed hydrolysis of pectin was investigated at the optimal pH for enzyme reaction observed at atmospheric pressure, that is, pH 7.2. As for thermal activation studies, an enzyme concentration of 14.5 units/mL and a pectin concentration of 0.4% were used.

The inactivation study revealed that pressures from 100 to 500 MPa protected PE from thermal inactivation at temperatures >40 ℃. In Figure 6, it is clearly illustrated that the enzyme not only is protected from thermal inactivation but even remains active for pressures up to 300 MPa. Corresponding estimated activities of tomato PE are summarized in Table 3. As at atmospheric pressure, the reaction rate at elevated pressure is dependent on temperature. The optimal temperature observed at elevated pressure shifted to higher values (respectively, 60–65 and 70 ℃ in the absence and presence of Ca^{2+} ions) as compared to atmospheric pressure (55 ℃).

At 100 and 200 MPa, a higher enzyme activity was observed in the temperature domain 50–70 ℃, as compared to atmospheric pressure, whereas at 300 MPa, only at temperatures >60 ℃ was a higher enzyme activity noted. As a consequence of these two tendencies (i.e., an increase in optimal temperature and an acceleration of the reaction under pressure), the catalytic activity of PE at temperatures >60 ℃ is significantly greater at pressures up to 300 MPa as compared to atmospheric pressure.

Table 3. Activity of Tomato PE at Elevated Pressure in the Absence and Presence of Ca^{2+} Ions as a Function of Temperature

	activity of tomato PE (mg of MeOH/mL of pectin solution, min)					
	absence of 60 mM Ca^{2+} ions			presence of 60 mM Ca^{2+} ions		
temp (°C)	100 MPa	200 MPa	300 MPa	100 MPa	200 MPa	300 MPa
50	3.99 ± 0.18[a]	4.05 ± 0.11	2.85 ± 0.15	ND[b]	ND	ND
55	5.06 ± 0.37	5.16 ± 0.27	3.23 ± 0.11	ND	ND	ND
60	5.20 ± 0.54	5.67 ± 0.18	4.48 ± 0.35	6.05 ± 0.12	6.58 ± 0.29	6.24 ± 1.15
65	5.82 ± 0.26	5.82 ± 0.39	4.15 ± 0.23	6.69 ± 0.41	7.66 ± 0.72	7.18 ± 0.57
70	4.08 ± 0.27	4.77 ± 0.21	ND	7.03 ± 0.79	9.42 ± 0.50	8.48 ± 0.58
75	ND	ND	ND	5.44 ± 0.49	7.20 ± 0.51	6.95 ± 0.31

[a] Standard errors are shown. [b] ND, not determined.

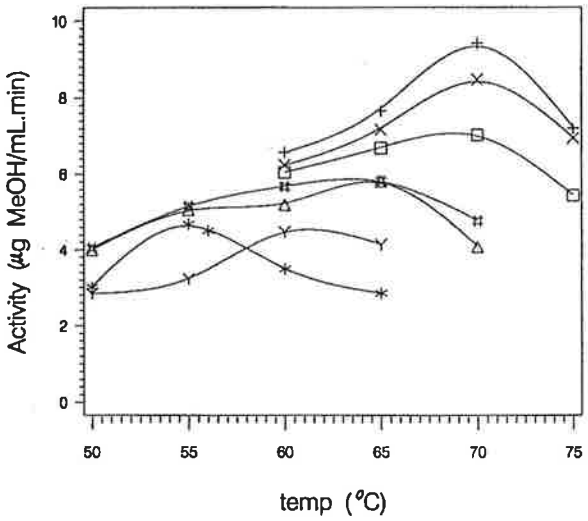

Figure 6. Enzyme activity as function of temperature at atmospheric pressure (*), 100 MPa (△), 200 MPa (#), 300 MPa (Y), 100 MPa and 60 mM Ca^{2+} (□), 200 MPa and 60 mM Ca^{2+} (+), and 300 MPa and 60 mM Ca^{2+} (×).

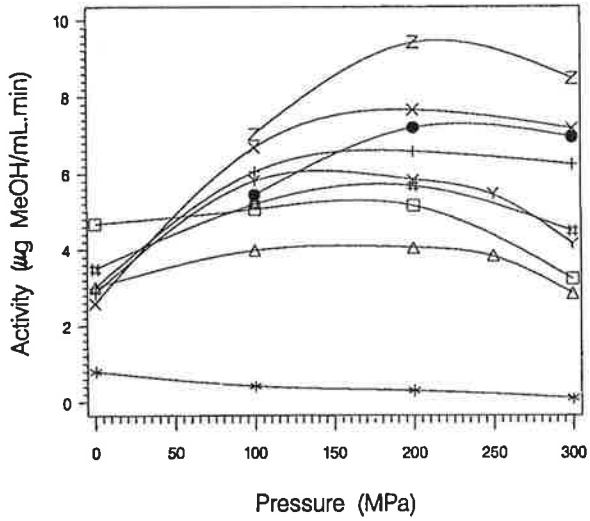

Figure 7. Enzyme activity as function of pressure at 20 °C (*), 50 °C (△), 55 °C (□), 60 °C (#), 60 °C and 60 mM Ca^{2+} (+), 65 °C (Y), 65 °C and 60 mM Ca^{2+} (×), 70 °C and 60 mM Ca^{2+} (Z), and 75 °C and 60 mM Ca^{2+}(●).

The presence of Ca^{2+} ions even increased the enzyme activity at elevated pressure up to 300 MPa and shifted the optimal temperature to 70 °C (see Figure 6). This is opposed to atmospheric pressure, at which the activity of PE decreased in the presence of Ca^{2+} ions and no shift in temperature optimum was observed.

In Figure 7 the effect of pressure on enzyme activity is visualized for the different temperatures and environments studied. From this figure it can be derived that for temperatures <55 °C, which was determined as the optimal temperature at atmospheric pressure, pressure up to 300 MPa has almost no influence on the enzymatic activity.

However, at temperatures at which at atmospheric pressure tomato PE inactivates (i.e., $T > 55$ °C), a clearly enhancing effect of pressure could be observed. The reaction accelerated with increasing pressure up to 100−200 MPa, at which the enzyme activity showed a maximum. At higher pressures, the rate of pectin hydrolysis, as measured by the formation of MeOH, diminished, although the activity was still higher as compared to atmospheric pressure.

The effect of pressure on the enzyme reaction was even more pronounced in the presence of Ca^{2+} ions (60 mM). The enzyme activity clearly increased with increasing pressure. The optimal pressure for the enzymatic reaction was situated between 200 and 300 MPa, which is 100 MPa above the optimal pressure found in the absence of Ca^{2+} ions.

Treatments that increase PE activity, such as temperature and pressure, may be interesting for improving the texture of fruits and vegetables. In the context of texture, another pectic enzyme, namely, PG, plays an important role. As mentioned in the Introduction, partially deesterified pectin can easily be depolymerized by PG, resulting in a dramatic decrease of consistency of products. It may be possible to design treatments that minimize unwanted degradation by PG and maximize reactions such as PE activity that lead to tissue firming by modifying the pectin structure (Eshtiaghi et al., 1994; Knorr, 1995). Recently, the production of canned tomatoes has been patented (Wilding and Woolner, 1997). Studies showed that, under suitable high-pressure conditions, PG was inactivated, whereas PE remained active, in contrast to thermal treatments, where PE is inactivated at lower temperatures than PG. In the absence of PG to depolymerize the pectic and pectinic acids formed by the action of PE on pectin, these acids remain in the cell wall to prevent structural breakdown. The selective inactivation of PG may therefore prevent texture degradation. Moreover, the removal of methyl ester groups from pectin molecules catalyzed by PE results in pectin molecules being able to associate with each other via cation cross-links to provide an increase in consistency (Wilding and Woolner, 1997). However, as the activation of PE at elevated pressure is more pronounced than at atmospheric pressure, care should be taken to avoid a too enhanced deesterification of pectin and, consequently, the production of uncookable products.

CONCLUSIONS

Tomato PE could be inactivated in the temperature range 57–65 °C at atmospheric pressure. However, a combined treatment at elevated pressure and 60 °C resulted in reduced inactivation as compared to atmospheric pressure. Pressure seemed to exert a protective effect on heat inactivation. Even at 900 MPa and 60 °C, the inactivation was slower as compared to the same treatment at atmospheric pressure.

The enzyme activity of tomato PE was influenced by extrinsic (temperature and pressure) and intrinsic (pH and Ca^{2+} ions) factors. At atmospheric pressure, the highest enzyme activity was noted at neutral pH and a temperature of 55 °C. At both lower and higher pH values, the enzymatic catalysis slowed and the temperature optimum shifted. Also, addition of Ca^{2+} ions (60 mM) decreased the enzymatic activity.

Increase of pressure up to 300 MPa, on the other hand, proved to be very efficient in stimulating catalytic activity at elevated temperature. At elevated pressure, the optimal temperature for enzymatic reaction shifted to higher values (60–65 °C). A combination with a pressure of 100–200 MPa seemed to be optimal. As opposed to atmospheric pressure, the activity of tomato PE under pressure increased in the presence of Ca^{2+} ions (60 mM), and the temperature optimum as well as the pressure optimum for maximal catalytic activity shifted to higher values, respectively, 70 °C and 200–300 MPa.

These results illustrate the potential of using high pressure for increasing the activity and stability of enzymes.

NOTATION

A	activity (μg of MeOH/mL of pectin, min)
A_t	activity at time t (mL/min)
A_0	initial activity (mL/min)
D	decimal reduction time (min)
D_{ref}	decimal reduction time at a reference temperature (min)
E_a	activation energy (kJ/mol)
k	first-order inactivation rate constant (min^{-1})
k_{atm}	first-order inactivation rate constant at atmospheric pressure (min^{-1})
k_{ref}	first-order inactivation rate constant at a reference temperature (min^{-1})
P	pressure (MPa)
P_{ref}	reference pressure (MPa)
R_p	universal gas constant (=8.314 cm^3·MPa/K·mol)
R_t	universal gas constant (=8.314 J/K·mol)
t	time (min)
T	temperature (K)
T_{ref}	reference temperature (K)
V_a	activation volume (cm^3/mol)
z_t	z value for thermal processing (°C)
z_p	z value for high-pressure processing (MPa)

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Received for review June 1, 1999. Revised manuscript received December 8, 1999. Accepted December 16, 1999. This research has been supported by the Flemish Institute for the promotion of scientific−technological research in industry (IWT) and the European Commission (Project FAIR-CT96-1175).

JF990569N