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Ultrasonication for tomato pectinmethylesterase inactivation: effect of cavitation intensity and temperature on inactivation

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Abstract

Ultrasonic inactivation tests with tomato pectinmethylesterase (PME) were conducted at cavitation intensities in the range of 0.004–0.020 mg L min^{-1} (hydrogen peroxide yield rate) and at 50, 61, and 72 °C. Thermal only tests at 50, 61, and 72 °C were also conducted to delineate possible additive or synergistic effects. In thermal inactivation tests, the reduction in PME residual activity at 50 °C was negligible while *D*-values at 61 and 72 °C were 299.0 and 25.3 min. *D*-values varied from 24.0 to 240.6 min in sonication tests but were reduced to 0.3 min in the thermosonication test at 72 °C and cavitation intensity of 0.008 mg L^{-1} min^{-1}. Compared to the PME thermal inactivation test at 61 °C, thermosonication at the same temperature increased the inactivation by 39 to 374-fold, while at 72 °C the increase was 36–84-fold, depending upon cavitation intensity. Generally, the inactivation increased with temperature and cavitation intensity. A strong synergistic effect was observed in the thermosonication tests. The increase in the inactivation was more pronounced at low temperatures. The inactivation of tomato PME in all tests exhibited first order kinetics.

Keywords: Pectinmethylesterase; Tomato; Thermosonication; Ultrasound; Cavitation

1. Introduction

The application of power ultrasound in food enzyme inactivation has been explored in recent years. Generally, ultrasonication in combination with other treatment(s) is more effective in enhancing the inactivation efficacy. Ordóñez, Sanz, Hernández, and López-Lorenzo (1984) explored the effect of combining heat with power ultrasound (thermosonication) and found that the microbial inactivation of thermosonication was greater than the sum of the inactivating effects of heat and ultrasound when acting independently. De Gennaro, Cavella, Romano, and Masi (1999) also used thermosonication as a means for peroxidase inactivation. The concept of combination treatment has been further explored by introducing elevated static pressure in an ultrasound treatment chamber in a process called manothermosonication (MTS). Manothermosonication has been used to deactivate lipoxygenase (López & Burgos, 1995a), peroxidase (López & Burgos, 1995b), lipase and protease (Vercet, Lopez, & Burgos, 1997), and tomato or orange pectinmethylesterase (Kuldiloke, 2002; López, Vercet, Sánchez, & Burgos, 1998; Vercet, López, & Burgos, 1999; Vercet, Sánchez, Burgos, Montanes, & López-Buesa, 2002), all with an increased inactivation. For example, López et al. (1998) reported that the *D*-value of tomato PME at 62.5 °C was reduced 53-fold, from 45 min in thermal treatments to 0.85 min by MTS.

The inactivation effect of ultrasound is attributable mainly to a phenomenon called cavitation. Cavitation

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refers to the formation, growth, and implosion of tiny gas bubbles or cavities in a liquid when ultrasound travels through it. Extreme physical phenomena (1000 K and 500 MPa) at micro-scale take place when the bubbles collapse (Suslick, 1988), and these phenomena are considered to be the cause of enzyme inactivation. The stable cavitating bubbles interacting with the acoustic field generate strong micro-streaming and high shear, which also contribute to the observed enzyme inactivation. In previous studies, efforts have been made to understand the effects of ultrasound frequency, amplitude, treatment time, temperature, and power density on inactivation rate. However, the role of cavitation activity, the fundamental cause for enzyme inactivation, remains largely unexplored. Cavitation studies have been restricted to the areas of heat transport, liquid tensile strength, and superheating and boiling phenomena (Apfel, 1981; Rooney, 1981).

In this study, pectinmethylesterase (PME; E.C. 3.1.1.11) in tomato juice is used in inactivation tests. PME is an endogenous pectic enzyme found primarily in tomato cell walls that de-esterifies the methyl group of pectin and converts it into low methoxy pectin or pectic acids (Giner et al., 2000). The low methoxy pectin or pectic acids can easily be depolymerized and hydrolyzed by polygalacturonase (PG), resulting in viscosity loss in tomato-based products. To avoid quality losses, partial or complete inactivation of PME together with inactivation of PG during tomato processing is required (Porreta, 1996). Cold break (<71 °C) is a thermal treatment that partially inactivates pectic enzymes in tomatoes and yields a paste of good color and taste quality. A problem associated with cold-break products is the instability of the viscosity during storage and relatively low consistency (Krebbers et al., 2003). In a "hot-break" process, tomatoes are quickly heated to temperatures in the range of 82–104 °C to completely deactivate enzymes (Gould, 1992). The drawback of the hot-break process is that the exposure of tomatoes to elevated temperatures leads to flavor losses, brown color and degradation of nutritional quality. Thermal treatment has also been found to lead to pastes with reduced pectin integrity (Gould, 1992; Porreta, Birzi, Ghizzoni, & Vicini, 1995). To improve product quality, different non-thermal technologies, including power ultrasound, have been tested in recent years to deactivate tomato enzymes.

Tomatoes contain different types of PME isoenzymes, but their thermostabilities are similar (López, Sánchez, Vercet, & Burgos, 1997). Published heat resistance rates (*D*-values) of tomato PME vary as a result of variations in treatment conditions. The *D*-values of tomato PME in citrate buffer reported by López et al. (1997) range from 7.6 min at 66.4 °C to 0.20 min at 74.5 °C with a *z*-value of 5.1 °C, while the *D*-values obtained with different substrates by other researchers are much higher (De Sio et al., 1995).

The objectives of the present work are to examine the efficacy of power ultrasound on the inactivation of tomato pectinmethylesterase and to study the effects of cavitation activity and temperature on inactivation.

2. Materials and methods

2.1. Reagents

Pectin powder from citrus fruits, potassium iodide, molybdic acid ammonium salt tetrahydrate, sodium hydroxide, and potassium hydrogen phthalate were purchased from the Sigma Chemical Co. (St. Louis, MO). 10 N sodium hydroxide, hydrogen peroxide, and sodium chloride were obtained from the Fisher Chemical Co. (Fair Lawn, NJ). Chemicals used were of reagent grade.

2.2. Raw material

Tomatoes (*Lycopersicon esculentum* L, cv. Roma) were purchased from a local supermarket and stored at 4 °C before the enzyme extraction. Tomatoes had a pH of 4.21 ± 0.03, as measured with a Fisher Scientific pH meter (AR 15 Accumet®, Fisher Scientific, Hannover Park, IL), and a soluble solid content of 0.1 ($g\,g^{-1}$), as measured with a refractometer (ABBE-3L, Bausch & Lomb, Rochester, NY).

2.3. Preparation of pectinmethylesterase (PME)

PME was prepared following the method proposed by Hagerman and Austin (1986) with modifications. Tomatoes were washed and wiped dry before cutting into four pieces. Fifty gram tomato samples were homogenized in 100 mL of 8.8% (w/v) NaCl (4 °C) with a household blender at high speed for 15 s. The homogenate was stirred with a magnetic stirrer for 15 min and then centrifuged at 20,000 × *g* for 25 min at 2 °C with a RC-5C centrifuge (Sorval Instruments Inc., Dupont, Willington, DE). The supernatant was kept in 150-mL plastic cups and frozen at –18 °C until use, which occurred within 1 month of the PME extract preparation.

2.4. Pectinmethylesterase assay

PME activity was assayed by an acid–base manual titration that was based on the production of free carboxylic groups by PME during hydrolysis of a pectin solution at pH 7.5 and at 30 °C. The assay followed the procedures of Rouse and Atkins (1995) with modifications. The substrate saturation conditions were investigated to ensure measurement for PME activity within the range of activities studied. A substrate, 0.5% (w/v) solution of citrus pectin, was prepared in 0.15 M NaCl. Before the assay, 195 mL or 190 mL of the pectin

solution was equilibrated to 30 °C and the pH was adjusted to 7.5 with 0.1 N NaOH. The reaction was initiated by adding 5 and 10 mL PME solution for thermosonication and thermal treatments, respectively. The reaction was sustained at 30 °C for 10–25 min. The reaction mixture was titrated to pH 7.5 with 0.1 N NaOH, and the volume of NaOH used was recorded. A blank (PME solution boiled for 10 min) was used to subtract the blank values from the reaction values. The PME activity was defined as the milliequivalents of acid from pectin per minute per milliliter of PME solution at pH 7.5 and 30 °C, and was calculated using Eq. (1) (Basak & Ramaswamy, 1997).

$$\text{PME activity(unit)} = \Delta V \times N/(V_e \times t) \quad (1)$$

where ΔV is the volume of standardized NaOH solution used for titration; N is the normality of standard NaOH solution; V_e is the volume of PME solution added into the reaction mixture; and t is reaction time in min. Each PME activity measurement was duplicated.

2.5. Evaluation of cavitation activity

The cavitation intensity was estimated by measuring hydrogen peroxide (H_2O_2) formation in distilled water during sonication following a catalyzed colorimetric procedure (Mead, Sutherland, & Verrall, 1976). The average rate of H_2O_2 formation in 20 min sonication was used as an indication of cavitation intensity. Two-and-a-half milliliters of solution A [1 g NaOH, 33 g KI, and 0.1 g $(NH_4)_6Mo_7O_{24}H_2O$ in 500 mL H_2O] were mixed with 2.5 mL of solution B [10 g $C_8H_5O_4K$ in 500 mL H_2O] just before mixing with 5 mL insonated distilled water. The color intensity was measured as absorbance at 350 nm using a HP 8452 UV/VIS Spectrophotometer (Waldbronn, Germany). Distilled water without sonication was used as a blank. The concentration of H_2O_2 was calculated based on a standard curve that correlated H_2O_2 yield to color intensity as expressed by the optical density. The R^2 of the calibration curves was 0.99.

Distilled water (50, 100 or 200 mL) was placed in a glass vessel and treated with a VC 750 ultrasonic unit (Sonics & Materials, Inc., Newtown, CT) at 20 kHz and amplitude of 20 μm. For each sample volume, H_2O_2 formation at 50, 61, or 72 °C was determined. Temperatures during sonication tests were controlled to ±2 °C of the designated values by placing the vessel in a temperature controlled water bath with agitation. The beakers were sealed with parafilm to prevent water and vapor leaks during sonication. Two replications were conducted for each treatment.

The determination of H_2O_2 generation during an ultrasound treatment in a food system such as a PME solution is complicated by the presence of ions and other colloidal components. There is, to date, no reliable method to measure cavitation activity in a food system. To delineate cavitation activities among sonication treatments of PME solutions, the H_2O_2 yield in distilled water subjected to the same treatment conditions was measured and used as an indication of cavitation intensity levels among the treatments used in PME inactivation tests.

2.6. Inactivation treatments

PME inactivation at three temperatures (50, 61, or 72 °C) was conducted in thermal tests. At each temperature, a PME solution of 100 mL placed in a test tube was preheated in a water bath set to 93 °C for less than 30 s to reach the designated temperature and was then immediately transferred to a 150 mL glass vessel held in a circulating water bath preheated to the same temperature, i.e. 50, 61, or 72 °C. Timing was started at the moment when the sample was transferred to the vessel and the PME activity at that point was taken as the initial activity in the subsequent analysis. PME samples of 5 mL were removed at designated time intervals, cooled in ice water, and assayed for PME activity.

In sonication tests, PME solution at three elevated temperatures (50, 61, and 72 °C) was sonicated at cavitation levels between 0.004 and 0.012 mg L H_2O_2 min^{-1}. The cavitation levels among the treatments were differentiated using test results obtained with distilled water of the same volume, treated under the same temperature and sonication conditions. A PME solution of 50, 100 or 200 mL heated to a test temperature following the procedure in thermal tests was treated with a VC 750 ultrasonic unit (Sonics & Materials, INC., Newtown, CT) at 20 kHz, amplitude of 20 μm, and initial input power of 100 W. PME activity at time zero was measured and taken as the initial activity. At the predetermined time intervals, 5 mL PME solution was pipetted into a glass test tube, immediately cooled by immersion in ice water, and assayed for enzyme activity. Five millimeters distilled water was added back after each sampling to maintain a constant sample volume during sonication. The dilution caused by adding back the 5 mL water was considered in residual PME activity calculations in both thermal an de-sonication tests. Each treatment was performed in duplicate.

2.7. Enzyme inactivation parameters

A D-value, the time required to decrease the initial enzyme activity by 90%, was used in the kinetic studies, and was calculated from the negative reciprocal of the slope on an inactivation curve, as given by

$$\text{Log}(A/A_0) = -(1/D)t \quad (2)$$

where, A is the activity of PME at time t; A_0 is initial PME activity; and D is the D-value (decimal reduction

time). A z-value, the increase in temperature to cause 90% reduction in D-value, is also used to describe the temperature sensitivity of PME under different treatment conditions.

2.8. Statistical analysis

The experimental design was a factorial plan with 3 heating temperatures × 3 cavitation levels with two repetitions. Statistical analysis was carried out using SAS software (SAS Institute, Cary, NC). Duncan's multiple range was used for mean discrimination. Linear regression analyses were conducted using the SigmaPlot 2001 (SPSS Inc., Chicago. IL). Significance of differences was defined at $P < 0.05$.

3. Results and discussion

3.1. Cavitation measurement

The detection limit of hydrogen peroxide (H_2O_2) yield was $0.1\,mg\,L^{-1}$. Variations among measurements of H_2O_2 yield were within 4–5%. The sensitivity and repeatability of the H_2O_2 measurements demonstrated the effectiveness of the detection method used in this study. The H_2O_2 generation rates varied from 0.004 to $0.020\,mg\,L^{-1}\,H_2O_2\,min^{-1}$ with changes in sample volumes (Table 1). The hydrogen peroxide yields as influenced by treatment temperatures at three sample volumes are plotted in Fig. 1. The H_2O_2 yield data was fitted to an Arrhenius equation:

$$\begin{aligned} &\mathrm{Ln}(Y) = \mathrm{Ln}(A) - E_a/(RT) \\ &\mathrm{Ln}(A) = -22.10 + 0.04V \quad (R^2 = 0.91) \\ &E_a = 49.53 - 0.11V \quad (R^2 = 0.95) \end{aligned} \tag{3}$$

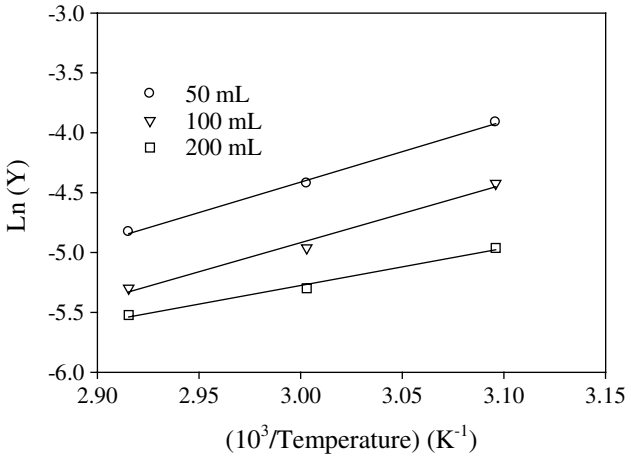

Fig. 1. Average hydrogen peroxide yield during sonication as a function of temperature at different treatment volumes.

where Y is H_2O_2 yield rate ($mg\,L^{-1}\,min^{-1}$); E_a is activation energy ($J\,mol^{-1}$); A is frequency factor; T is temperature (K); R is the gas constant ($R = 8.314\,J\,mol^{-1}\,K^{-1}$); and V is sample volume (mL). Since the acoustic power input during ultrasound treatments was maintained at about the same level, a sample with small volume had a higher volumetric power density. Higher H_2O_2 generation for samples with small volumes must be attributed to an increase in acoustic power density. H_2O_2 yield decreased as temperature increased. Although increasing liquid temperature during sonication allows a reduction in cavitation threshold, the maximum temperature and pressure when a cavitational bubble collapses will be decreased (Mason & Lorimer, 2002). In other words, the sonochemical reaction that generates H_2O_2, i.e., H_2O-)-)-) $\rightarrow OH^{\cdot} + H^{\cdot} \rightarrow H_2O_2 + H_2$, would be less intense at elevated temperatures (Suslick, 1986). As a result,

Table 1
Inactivation of PME in tomato juice with different methods and D-values under different treatment conditions

Method	Temperature (°C)	Treatment volume (mL)	Cavitation intensity ($mg\,L^{-1}\,min^{-1}$)	D-value[a] (min)	R^2
Thermal	50	100	–	1,571.4[a]	0.40
	61	100	–	299.0[b]	0.93
	72	100	–	25.3[c]	0.99
Sonication	50	200	0.007	240.6[g]	0.97
	50	100	0.012	42.7[d]	0.95
	50	50	0.020	24.0[j]	0.94
Thermo-sonication	61	200	0.005	7.6[h]	0.99
	61	100	0.007	1.5[e]	0.98
	61	50	0.012	0.8[k]	0.97
	72	200	0.004	0.7[i]	0.98
	72	100	0.005	0.4[f]	0.98
	72	50	0.008	0.3[l]	0.93

1. a, b, c, d, e, f = significant difference among thermal, sonication, and thermosonication treatment for sample volume of 100 mL ($P < 0.05$).
2. g, h, i = significant difference between sonication and thermosonication treatment for sample volume of 200 mL ($P < 0.05$).
3. j, k, l = significant difference between sonication and thermosonication treatment for sample volume of 50 mL ($P < 0.05$).

[a] The confidence interval used in D-value estimation is 95%.

the H_2O_2 yield and thereby the cavitation activity decreases with temperature.

3.2. Inactivation of tomato PME by thermal treatment

The residual PME activity (A/A_0) at three temperatures is shown in Fig. 2. The data fit well to a first order kinetic model, similar to that shown in the work of De Sio et al. (1995) and Crelier, Robert, Claude, & Juillerat (2001). From Table 1, it can be seen that at 50 °C, enzyme inactivation caused by heating is negligible, as indicated by a D-value of 1571.4 min. When the temperature was increased to 61 and 72 °C, the time needed to achieve 90% reduction in PME residual activity was reduced to 299.0 and 25.3 min, respectively. Increasing the temperature increased the inactivation rate, which has been demonstrated in many tomato enzyme thermal inactivation tests (Crelier et al., 2001; López et al., 1997). The come-up times of <30 s are not considered in D-value determinations. Compared to the magnitude of D-values in Table 1, the errors caused by this are small and negligible. The D-values reported by other researchers at various temperatures, together with D-values from this work, are tabulated in Table 2. At similar temperatures, for instance at 60 °C, D-values published by different groups significantly differ from each other. The D_{60} (15.17 min) for commercial tomato PME in distilled water reported by Van den Broeck, Ludikhuyze, Van Loey, & Hendrickx (2000) is one magnitude smaller than the values from tests with PME in tomato juice (present work and that of Crelier et al., 2001). From our data, the z-value of thermal inactivation is 12.3 °C ($R^2 = 0.99$), which is comparable to that of De Sio et al. (1995) but larger than others. The discrepancies might be caused by differences in tomato variety, degree of ripening, heating and temperature measurement techniques, and enzyme preparation and assay method (López et al., 1997). The solid matrix in the solution, pH, and existence and type of co-solutes also contribute to the observed disparity in the D and z-values (López et al., 1997; Tajchakavit & Ramaswamy, 1997). Generally, commercial and purified PME are more heat labile compared to crude PME.

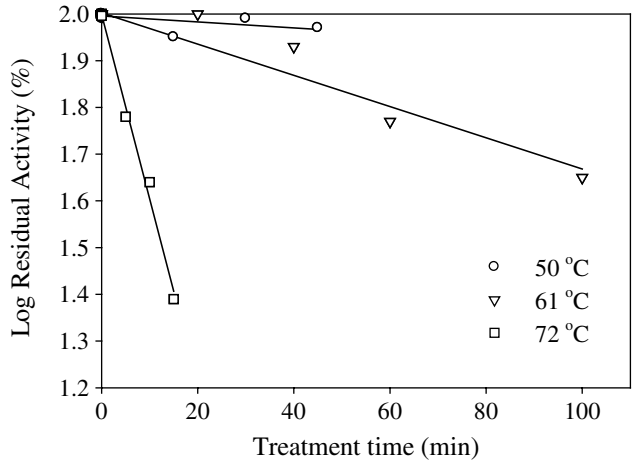

Fig. 2. Tomato PME inactivation by thermal treatment at three temperatures.

Table 2
Comparison of D and z-values in tomato PME inactivation by thermal treatment

D-value (min)	z-value (°C)	Substrate	Reference
$D_{78–88} = 0.2$	11.2 (78–88)	Commercial tomato PME	De Sio et al. (1995)
$D_{66.4} = 7.6$ $D_{70.5} = 1.3$ $D_{74.5} = 0.2$	5.1[a]	Purified tomato PME	López et al. (1997)
$D_{57} = 37.4$ $D_{60} = 15.2$ $D_{63} = 4.7$ $D_{65} = 2.2$	6.5	Commercial tomato PME in distilled water	Van den Broeck et al. (2000)
$D_{60} = 103.5$[a] $D_{65} = 19.1$ $D_{70} = 2.7$ $D_{75} = 0.5$	6.4[a]	PME in tomato juice	Crelier et al. (2001)
$D_{50} = 1571.4$ $D_{61} = 299.0$ $D_{72} = 25.3$	12.3	PME in tomato juice	This work

[a] Values are estimated from data reported in the original publication.

3.3. Inactivation of tomato PME by ultrasonication at 50 °C (sonication)

The efficacy of ultrasonication on tomato PME inactivation was investigated at 50 °C and cavitation levels between 0.007 and 0.020 mg L^{-1} min^{-1}. Resulting D-values and the corresponding regression coefficients (R^2) are summarized in Table 1. It is obvious that increasing cavitation intensity increases inactivation. Since $D_{50} = 1571.4$ min for thermal treatment at 50 °C, in the time frame of the inactivation tests, i.e., <80 min, the inactivation caused by heating at 50 °C is negligible. Therefore, at 50 °C when the temperature is not high enough to cause a decrease in PME activity, PME inactivation in an ultrasound treatment at 50 °C is due to sonication itself. Comparing thermal inactivation with sonication tests, one can see that sonication substantially decreases tomato PME activity at 50 °C (Fig. 3). The D-value decreased from 1571.4 min for 0.0 mg L^{-1} min^{-1} to 24.0 min for a cavitation intensity of 0.020 mg L^{-1} min^{-1}. To examine the effect of sonication on PME inactivation, the D-value at 50 °C due to heat treatment is compared to D-values due to sonication at the same temperature. When cavitation activity

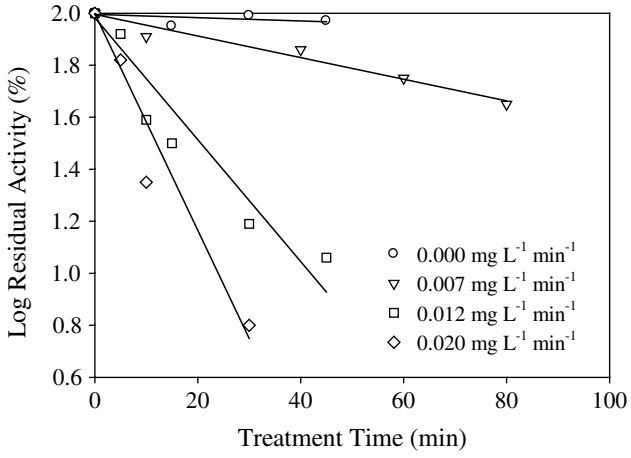

Fig. 3. Effect of cavitation intensity on tomato PME inactivation by ultrasonication (20 kHz, amplitude of 20 μm) at 50 °C.

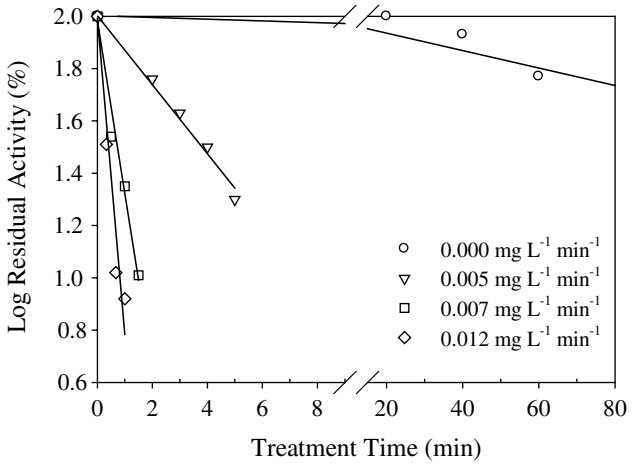

Fig. 4. Effect of cavitation intensity on tomato PME inactivation by ultrasonication (20 kHz, amplitude of 20 μm) at 61 °C.

is relatively low ($0.007\,mg\,L^{-1}\,min^{-1}$), the $D_{thermal}/D_{sonication}$ is 7, indicating a 7-fold increase in inactivation when ultrasound is applied. This ratio increases to 53 at a higher H_2O_2 yield ($0.012\,mg\,L^{-1}\,min^{-1}$), which demonstrates that increasing cavitation intensity substantially increases the inactivation.

Effects of ultrasound on enzymes are often ascribed to several mechanical and sonochemical processes induced by cavitation (Price, 1992). The microjets of liquid generated by the asymmetrical collapse of cavitation bubbles, the shear stress in a sonicating liquid, and the microstreaming caused by stable oscillating bubbles might mechanically damage the integrity of the PME protein structure and causes loss in PME activity. The high pressure gradient could cause fragmentation of protein molecules or other structural modifications while the large temperature gradient may lead to thermal inactivation of the protein or pyrolysis of bonds in the protein (Krishnamurthy, Lumpkin, & Sridhar, 2000). Obviously, at high cavitation intensity levels there will be more damage to the PME structure, resulting in a higher inactivation no matter what mechanisms might be involved.

3.4. Inactivation of tomato PME by ultrasonication at 61 and 72 °C (thermosonication)

PME inactivation curves at 61 and 72 °C are shown in Figs. 4 and 5 and the corresponding *D*-values are listed in Table 1. Similarly, inactivation increased with increase in the H_2O_2 yield rate. Much faster inactivation can be observed from Figs. 4 and 5 compared to the thermal treatment and sonication at 50 °C. The *D*-values obtained from thermosonication at any observed temperature were much smaller than those for thermal and sonication inactivation. Inactivation by the combined action of heat and ultrasound can also be

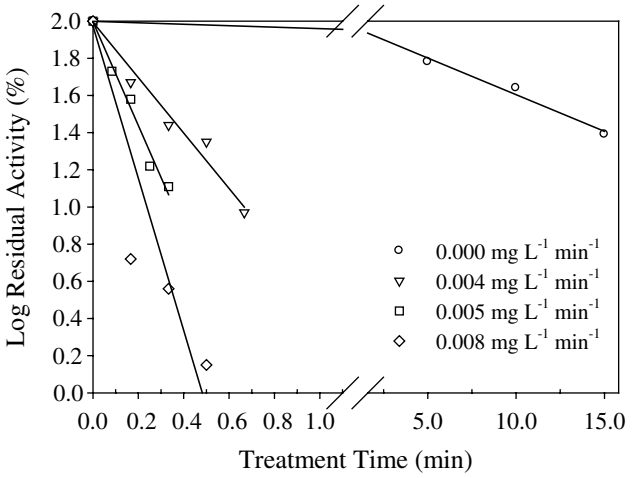

Fig. 5. Effect of cavitation intensity on tomato PME inactivation by ultrasonication (20 kHz, amplitude of 20 μm) at 72 °C.

examined with ratios of $D_{sonication}/D_{thermosonication}$ at the same cavitation level. The *D*-value for sonication at 50 °C was 240.6 min ($0.007\,mg\,L^{-1}\,min^{-1}$). When the temperature was increased to 61 °C to introduce heat-induced inactivation, the *D*-value was reduced to 1.5 min at the same cavitation level of $0.007\,mg\,L^{-1}\,min^{-1}$. The 160-fold increase (D_{50}/D_{61} at $0.007\,mg\,L^{-1}$ cavitation intensity) must be caused by the added effect of heat inactivation. This ratio was reduced to 53 at higher temperatures (D_{50}/D_{72} at $0.012\,mg\,L^{-1}$ cavitation intensity), although the cavitation intensity was higher ($0.012\,mg\,L^{-1}\,min^{-1}$). It seems that the increase in inactivation in thermosonication is more pronounced at lower temperatures. One possible explanation could be that at higher temperatures, increased vapor pressure inside the bubbles introduces a cushioning effect and hence produces less effective collapses (Mason, 1990). By summarizing the work of several research groups on PME

inactivation, Crelier et al. (2001) also noticed that PME is more heat sensitive at low temperatures. The z-value for the thermosonication tests is 8.6 °C ($R^2 = 0.93$). Compared to that in the thermal tests (12.3 °C, $R^2 = 0.99$), it can be seen that the combined action of heat + ultrasound made the PME more labile to heat treatment.

It is of interest to discuss the effect of combined heat and ultrasound treatment on PME inactivation. Fig. 6 shows a comparison among PME inactivation by thermal treatment (61 °C), sonication (50 °C + ultrasound at 0.012 mg L^{-1} cavitation intensity), and thermosonication (61 °C + ultrasound at 0.012 mg L^{-1} cavitation intensity). The fourth curve (the dotted line) in Fig. 6 represents the situation in which inactivation caused by thermal treatment and that by sonication is additive. The fourth curve is given by

$$\begin{aligned} \Lambda &= \Lambda_1 + \Lambda_2 \\ \Lambda_1 &= 2.00\text{–}3.34 \times 10^{-3} t \quad (R^2 = 0.93) \\ \Lambda_2 &= 1.98\text{–}2.34 \times 10^{-2} t \quad (R^2 = 0.92) \end{aligned} \tag{4}$$

where Λ_1 is the linear regression equation for the thermal inactivation data; Λ_2 is the one for sonication; Λ is the one for the additive effect curve; and t is time (min). As one can see from Fig. 6, there was a synergistic effect for the combined action of heating at 61 °C plus sonication with a much higher inactivation compared to the additive curve. To analyze the synergistic effect, a parameter, the synergistic enhancement ratio is used in this study. It is defined as the time used to cause a 90% reduction on an additive effect curve over the time used to cause a 90% reduction on the experimental curve.

From Fig. 6, this ratio is 36 min/0.8 min = 45, indicating a strong synergistic effect between the heat treatment and the ultrasound treatment at the same temperature.

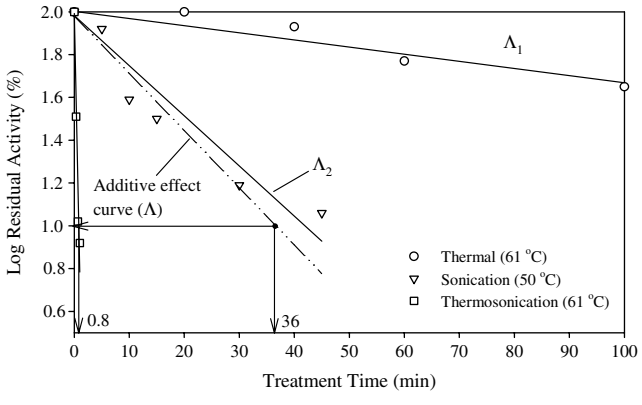

Fig. 6. Inactivation curves for tomato PME at cavitation level of 0.012 mg L^{-1} min^{-1}: Λ_1, thermal treatment alone (61 °C); Λ_2, sonication at 50 °C in which inactivation caused by heat is negligible; Λ, additive effect curve (61 °C). For sonication and thermosonication tests, the cavitation level was both at 0.012 mg L^{-1} min^{-1}.

This approach of analyzing a combined treatment has been used by Petin, Zhurakovskaya, and Komarova (1999) to identify the synergistic effect for the simultaneous action of ultrasound and hyperthermia on yeast cells. Although the synergistic effect of combining heat with power ultrasound (thermosonication) has been well documented by Ordóñez et al., 1984, Ordóñez, Aguilera, García, and Sanz (1987), and Wrigley and Llorca (1992) in microbial inactivation tests, few have studied the effect of the combination of heat with ultrasound on enzyme inactivation. The mechanism under which a synergistic action takes place in PME thermosonication is not clear. More studies are needed to understand and better utilize this phenomenon.

4. Conclusions

Ultrasound treatment effectively increased the tomato PME inactivation compared to a thermal treatment at the same temperature. When sonication was combined with a heat treatment at temperatures high enough to cause thermal inactivation, a significant synergistic effect was observed. Tomato PME inactivation exhibited first order kinetics in all treatments. In ultrasound treatments, the PME inactivation increased with an increase in the cavitation intensity expressed by H_2O_2 yield.

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