Thermal Inactivation of Pectin Methylesterase in Tomato Homogenate

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Abstract

It is well known that pectin methylesterase (PME) is one of the most important enzymes affecting the quality of processed tomato products. A number of investigators have studied thermal inactivation of tomato PME using partially or fully purified enzymes. The aim of the present work was to calculate D and z values for thermal inactivation of PME in the homogenates among tomato cultivars growing in the U.S. and Japan, which will more closely represent actual processing conditions.

D and z values for thermal inactivation of PME were determined in five U.S. and seven Nippon Del Monte (NDM) cultivars. The enzyme was comprised of both resistant and labile isozymes. At 70°C, the D value for labile and resistant isozymes of U.S. cultivars ranged from 5.0 to 9.5 min and 12.0 to 13.9 min, respectively. The z value of labile and resistant isozymes ranged from 4.9°C to 5.9°C and 5.1°C to 6.3°C, respectively. At 70°C, the D value of the labile and resistant isozymes of NDM cultivars ranged from 5.8 to 9.6 min and 10.1 to 13.9 min, respectively. The z value of labile and resistant isozymes ranged from 5.9 to 6.8°C and 6.2°C to 7.6°C, respectively.

D value of cold-break cultivar did not essentially differ from that of hot-break cultivar. D values determined in homogenate were higher than those reported for purified PME performed in buffer solutions. This suggests that controlling thermal processing is important in the production of tomato paste or diced tomatoes.

INTRODUCTION

Pectin methylestrase (PME), which is widely distributed in higher plants, is attributed to drastic changes in pectin during ripening. This enzyme plays also an important role in the degradation of pectins in the primary cell wall and middle lamella of tomato fruit during the processing. After PME catalyzes the deestrification of pectin, yielding a uronic acid carboxyl and methanol, polygalacturonase (PG) cleaves the polygalacturonic acid chain of the pectin, which results in the reduction of tomato juice viscosity (Eskin, 1979). Consequently the thermal inactivation of PME is an important from a tomato processing point of view. However, there were substantial differences in inactivation parameters of PME using an extracted enzyme or tomato homogenate, which have been reported in the literature (De Sio et al.,1995, Laratta et al.,1995, Castaldo et, al.,1996). Additionally the influences of tomato cultivars growing in different areas on thermostability of PME are not available in published papers. Therefore, for thermal inactivation of PME, the aim of present study was to examine D and z values in tomato homogenate, and to investigate the variations between American and NDM cultivars, and to determine the influence of pH.

MATERIALS AND METHODS

Tomatoes

Five American cultivars, which were harvested at maturity in 1999, were obtained from seed and paste processing companies in California. Seven Japanese cultivars grew in 2000 were harvested at the ripened stage in the test field of Nippon Del Monte. Tomatoes were washed, sliced to remove the seeds and locular gel, then cut into a half-inch dice, which were stored in PE bags at –80°C upon the use after freezing in a –80°C blast freezer

or in liquid nitrogen.

Inactivation Studies

Followed by thawing diced tomatoes, homogenates grinded in a small Waring blender for 30 seconds were passed through a 16-mesh screen to remove peels. The homogenate was transferred into glass tubes using a syringe with a blunt needle. The glass tubes were immersed in water bath at different temperatures for given time, cooled rapidly in ice water, and then held on ice until assay. Two kinds of thin-walled glass tubes with the capacity of 200μL(1.5mm i.d.x125mm length) or 1mL(4.8mm i.d.x 105mm length), which were open at one end and sealed at the other, were used for heat inactivation. The crude PME was extracted by the procedure of Pressey and Avants (1972).

PME Assay

PME activity was assayed according to the titrimetrical procedure of Lee and MacMillan(1968). Citrus pectin solution at 1.0%(w/w) in 0.2 M NaCl was used as a substrate. The PME activity was expressed in PE U (micromoles of acid produced per minute per mL of homogenate).

RESULTS AND DISCUSSIONS

Kinetic Studies on Thermal Inactivation of PME in Tomato Homogenate

The thermal inactivation of tomato PME in the homogenate from Roma cultivar was determined in a temperature ranged from 69.8°C to 83.8°C. As shown in Figure 1, the plot of the log residual PME activity could be exhibited a biphasic pattern. The same behavior was obtained in all other cultivars investigated. Figure 2 showed the thermal inactivation curve of both phases of PME, thermolabile and resistant, from Roma cultivar. From this curve, z values of both phases were calculated. This might demonstrate the presence of different thermoresistant PME isoforms in tomatoes as reported by De Sio et al. (1995) and Laratta et al. (1995).

Thermal Stability of PME among Tomato Cultivars Growing in the U.S. and Japan

Variations of the thermal inactivation parameters D and z for all cultivars were indicated in Table 1. At 70°C for thermolabile phase, PME from NDM 974 was the most stable at 9.6 min, while the enzyme from H 9492 at 5.0 min showed the least heat stability with the other cultivars ranging from 5.8 to 9.5 min. For resistant phase at the same temperature, the highest heat resistance was found with CXD 153 and NDM 153, and the lowest with NDM 843. The thermal inactivation data of CXD 199 for processing cold break paste did not essentially differ from that of tomato cultivar, BOS 3155, being used for the production of hot break paste. Several researches have conducted kinetic studies of thermal inactivation for purified or crude PMEs and have found substantial differences in D values (De Sio et al.,1995; Laratta et al.,1995; Lopez et al.,1997; Van den Broeck et al.,2000; Crelier et al.,2002; Hui et al.,2002). This may be due to the nature of PME, purity of the enzyme and experimental procedures. As shown in Table 1, the largest z value was in thermolabile PME from NDM 447 and 737 and themoresistant PME from NDM 153, whereas the smallest z value was found in CXD 152 in thermolabile and CXD 152, and H 9492 in thermoresistant. The range of z values for the other cultivars was between 5.0°C and 6.3°C in thermolabile PME and between 5.2□ and 6.9□ in thermoresistant PME. The z values obtained in this study were in agreement with the literature values reported by Lopez et al. (1997), Van del Broeck et al. (2000), and Hui et al. (2002).

De Sio et al.(1995) reported the thermal inactivation of PME in five cultivars of tomato growing in Italy, which indicated the limited variation. In accordance with this, there was no fundamental difference in the inactivation kinetics of PME between American and NDM cultivars.

Influence of pH on Thermal Stability of PME

The effect of pH range 4-6 on the thermal stability of crude PME extracted from

CXD 199 was determined. As shown in Figure 3, the PME had maximum thermal stability at pH 5.0 and its thermal resistance was influenced by pH in the medium. For instance, the changes in pH from 5.0 to 4.0 resulted in decreasing D value, from 3 to 0.45 min. Svensson and Eriksson (1972) demonstrated that the D value of lipoxygenase from peas reduced drastically when the enzyme was heated in acidic pH. In addition, the D value (4.1 min) at the temperature 71.8°C heated in homogenate, pH 4.36, differed from the estimated $D_{71.8}$ (1.5 min) of crude enzyme. This indicates that thermal inactivation data of PME obtained from investigations on tomato homogenate are very valuable for processing tomato industry since these data include the influence of microenvironments including pH of the enzyme on thermostablity.

CONCLUSION

The D_{70} and z values of thermolabile PME in tomato homogenate were 5.0-9.6 minutes and 4.9-6.8°C, respectively. The PME activity indicates to be susceptible to the temperature. The difference in D and z values between U.S. and NDM cultivara was not essentially observed. The thermostability of PME was influenced by pH and medium in heat treatment. Therefore, thermal inactivation parameters of PME obtained in tomato homogenate should closely represent actual processing conditions.

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Tables

Table 1. D and z values for thermal inactivation of tomato PME in homogenate.

Thermolabile		Thermoresistant		
Cultivar	D_{70} (min)	z (°C)	D_{70} (min)	z (°C)
Roma	5.9	5.9	13.6	6.3
CXD 152	9.3	4.9	13.9	5.1
CXD 199	9.5	4.9	13.5	6.1
H 9492	5.0	5.6	12.0	5.1
BOS 3155	6.6	5.2	12.7	5.2
NDM 051	5.8	6.3	11.4	6.6
NDM 153	8.9	6.2	13.9	7.6
NDM 447	5.8	6.8	12.9	6.4
NDM 737	6.0	6.8	13.5	6.9
NDM 843	6.5	5.9	10.1	6.3
NDM 973	6.4	6.3	11.0	6.5
NDM 974	9.6	6.0	13.6	6.2

Figures

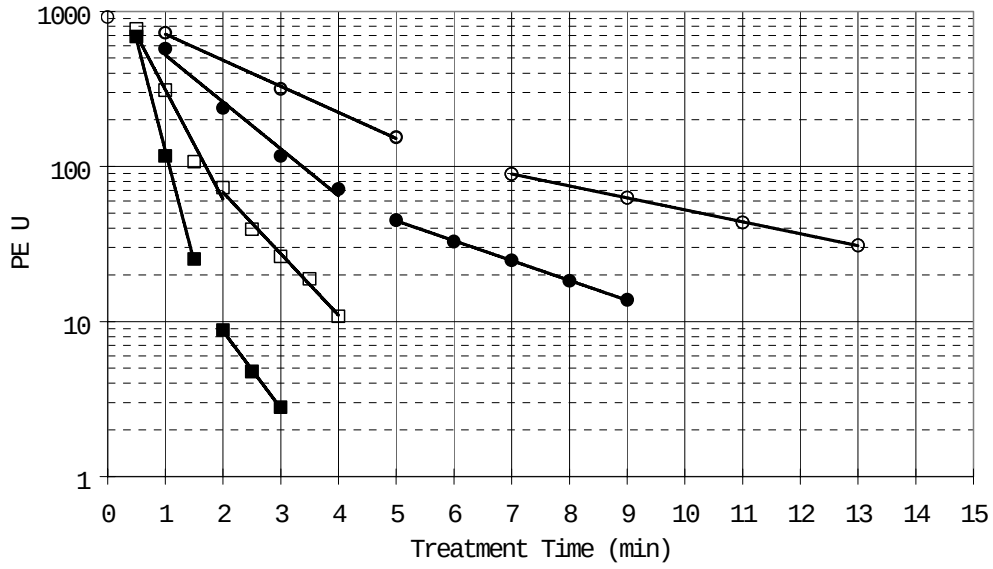

Fig. 1. Thermal inactivation of tomato PME from Roma cultivar at 69.8(○), 71.8(●), 73.8(□), and 75.8(■)□.

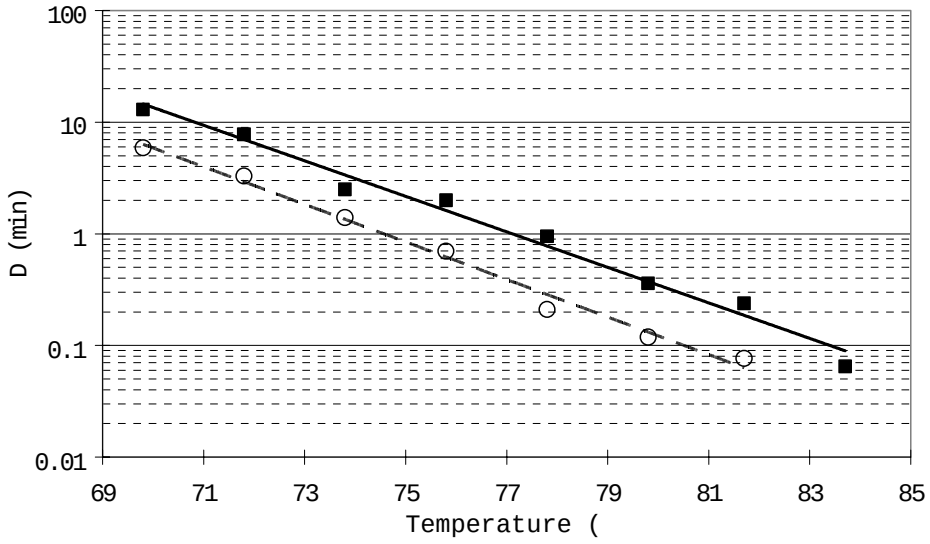

Fig. 2. Thermal inactivation curve of tomato PME from Roma cultivar as a function of temperature. Labile(○) and Resistant(■).

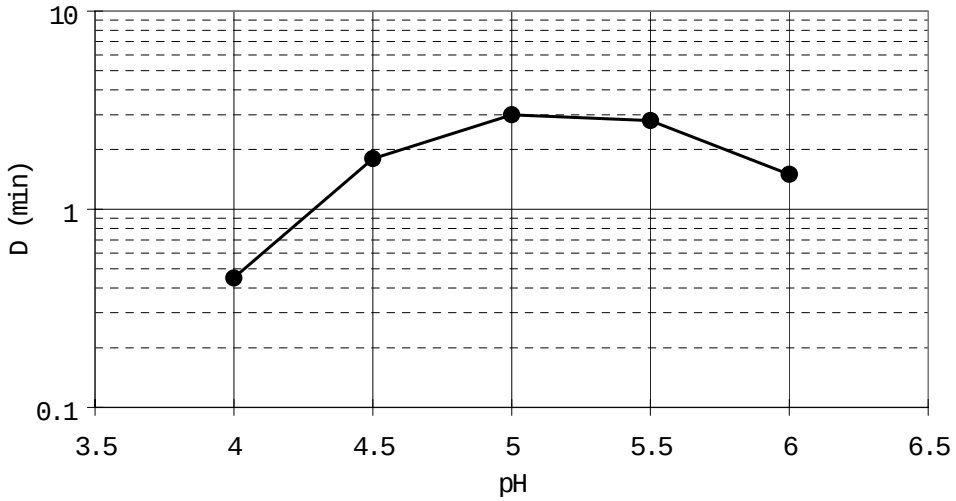

Fig. 3. Thermal inactivation of thermolabile crude PME extracted from CXD-199 as a function of pH. Temperature 71.8°C.

Data