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Soursop pectinesterases: thermostability and effect on cloud stability of soursop juice

S.M. Arbaisah, B.A. Asbi[a], A.H. Junainah, B. Jamilah and J.F. Kennedy[b]*

[a]*Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia, 43400 Serdang, Selangor, Malaysia*
[b]*Birmingham Carbohydrate and Protein Technology Group, School of Chemistry, University of Birmingham, Birmingham, B15 2TT, UK*

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The thermostability of two forms of purified pectinesterase from soursop fruit were studied. The heat stability data showed that PE I is more heat stable than PE II at pH 7.5. The *D* and *Z* values were evaluated in the range 45–75°C. The *D* values at 65°C were approximately 5.8 min and 3.3 min for PE I and PE II respectively. The changes in temperature required to increase the inactivation rate tenfold (*Z* value) were calculated at 8.5 and 8.6°C for PE I and PE II respectively. Both enzymes also tested positive for their ability to destabilise soursop juice cloud at 5 and 30°C. Cloud destabilisation by PE I occurred the fastest (large decrease in A 660 nm) in the natural juice at 30°C. Copyright © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Pectinesterase (PE) has been recognised as being one of the most heat resistant cloud destabilising enzymes in orange juice (Eagerman and Rouse, 1976; Korner *et al.*, 1980; Versteeg *et al.*, 1980; Marshall *et al.*, 1985; Wicker and Temelli, 1988). In many fruit processing industries it is important that the hydrolysis effects of pectinesterase are minimised, especially for juice cloud maintenance in citrus juice products. Many producers of fruit and nectar juices have complained of consistency loss occurring to as much as 50% in apricot and peach puree during storage in tanks (Giovane *et al.*, 1994). Sims *et al.* (1993) also reported that loss of cloud stability is one of the quality problems that limit the marketing of carrot juice. Papaya pectinesterase also has an important influence on the quality and stability of processed papaya products (Fayyaz *et al.*, 1994).

It has previously been determined that the thermal resistance of the common spoilage bacteria and yeasts occurring in citrus juices is less than that of pectinesterase (Bisset *et al.*, 1953; Patrick and Hill, 1957). Cameron *et al.* (1994) mentioned that to overcome this quality defect, citrus juices must be pasteurised at temperatures greater than needed to control microbial growth in order to inactivate the heat stable pectinesterase. Moreover, Laratta *et al.* (1995) noted that the thermal treatments generally employed in the food industry to ensure microbiological stability are at the lower temperature necessary to inactivate pectinesterase. Commercial citrus processors therefore generally inactivate these enzymes with high-temperature short-time pasteurisation.

However, the organoleptic qualities of juices may be severely damaged by this high heat treatment. Heat treatment of juices should therefore be limited to the minimum to prevent 'cooked' off-flavours in juice but at the same time should be strong enough for microbial inactivation. Hence, the adequacy of juice pasteurisation is dependent on a trade-off between cloud stability and pectinesterase inactivation (Nath and Rangganna, 1977).

Since the properties of different fruits vary, it is advisable to determine the optimum conditions for the processing of different fruits (Fayyaz *et al.*, 1995a). This study was conducted to examine the thermostability of soursop pectinesterase and its effect on cloud stability.

MATERIALS AND METHODS

Materials

Citrus pectin purchased from Fluka Chemical, Buchs, Switzerland, had a degree of esterification

*To whom correspondence should be addressed.

of 63%–66%. Potassium metabisulphate used was food grade.

Source of enzyme

The pectinesterase enzyme was previously extracted and purified according to the method established by Arbaisah *et al.* (1996a, b). The purified PE I and PE II enzymes that were used for thermostability study had specific activities of 81.7 μmol/min per mg and 439.20 μmol/min per mg respectively. The enzyme was kept in 0.02 M sodium phosphate buffer (pH 7.5) solution containing 0.2 M NaCl and 0.02% sodium azide at 4°C.

Pectinesterase activity determination

Pectinesterase activity was determined by the method of Kertesz (1955) as described by Fayyaz *et al.* (1993) using a Titralab Autotitrator model VIT 90/ABU 93/SAM 90 (Radiometer, Copenhagen). The pectinesterase activity was expressed in units where one unit is that amount of enzyme that will release 1 μmol of pectic acid produced per min at pH 7 and 30°C.

Juice preparation

Frozen soursop pulp prepared according to the method described by Arbaisah *et al.* (1996b) was thawed and homogenised in a ratio of 1:3 (w/v) of pulp to water using a Waring blender (Model 7011S) and filtered using muslin cloth. Potassium metabisulphate was added (1000 mg/litre) to preserve the juice. The pH of the juice was 3.7 with total soluble solids of 11.5° Brix. Heat-treated juice was prepared by heating fresh juice at 70°C for 5 min and subsequent cooling.

Thermal stability studies

Samples of pectinesterase of 300 μl of PE I and 50 μl of PE II were added to 15 ml Falcon plastic tubes containing of 1700 μl and 1950 μl of preheated 20 mM phosphate buffer (pH 7.5) respectively at various temperatures (10–90°C) and immediately capped, vortexed, stirred, and replaced in the water bath (time = zero). The samples were incubated at the selected temperature for 5 min. Following heat treatment, the tubes were cooled instantly at 1°C to minimise any further change in enzyme activity. The residual enzyme activity was measured under standard assay conditions.

***D* and *Z* values**

Pectinesterases of 0.5 ml of PE I and 0.3 ml of PE II were added to Falcon centrifuge tubes with 11.5 ml and 11.7 ml 0.02 m sodium phosphate buffer, pH 7.5, respectively. The mixtures were then treated as in the heat stability studies described above, but the incubation times at the different temperatures were varied. The log of per cent residual enzyme activity was plotted against incubation time to obtain the heat-effected enzyme activity destruction curve. The *D* values at the different temperatures were obtained from the slopes of the plot and used for thermal destruction curves. The *Z* values were next calculated from the slopes of thermal destruction curves.

Cloud stability determination

For cloud stability study, PE I and PE II which had activities of 3.24 units/ml and 12.52 units/ml respectively were added to the level of activity in fresh juice (measured to be approximately 3 units/ml). At intervals each sample was mixed thoroughly by inverting the bottle ten times. Aliquots (10 ml) were placed in graduated 15 ml conical centrifuge tubes and centrifuged for 10 min at 360 *g*. Supernatant (3 ml) was placed into a 10 mm glass cuvette and the absorption measured spectrophotometrically at 660 nm. After measurement this supernatant was returned to the centrifuge tube and mixed by shaking. The absorption values were plotted against incubation (storage time).

RESULTS AND DISCUSSION

Thermostability of PE I and PE II

The residual activity of the purified PE I and PE II after heating at various temperatures in phosphate buffer of pH 7.5 is shown in Fig. 1. The activity of PE I was substantially unaffected up to 50°C and then it decreased to about 20% at 60°C and was undetectable at 75°C. For PE II, it was found that there was a gradual drop in activity from 40°C to 50°C and this was followed by a relatively sharper drop from 55°C onwards. From Fig. 1 it can be seen that PE I was more heat stable than PE II in the buffer of pH 7.5. The purified PE I lost only 65% of its activity when held at 65°C for 3 min as compared to PE II which lost 90% activity when held at the same temperature.

According to published figures, the temperature of inactivation of plant pectinesterases (PE) shows a wide range. Seymour *et al.* (1991) studied the thermostability of Marsh white grapefruit pulp PE by heating for 5 min at various temperatures at pH 7.0 in 10 mM phosphate buffer. They observed that thermolabile PE and thermostable PE were inactivated completely at 65 and 85°C respectively. Versteeg *et al.* (1980) reported that

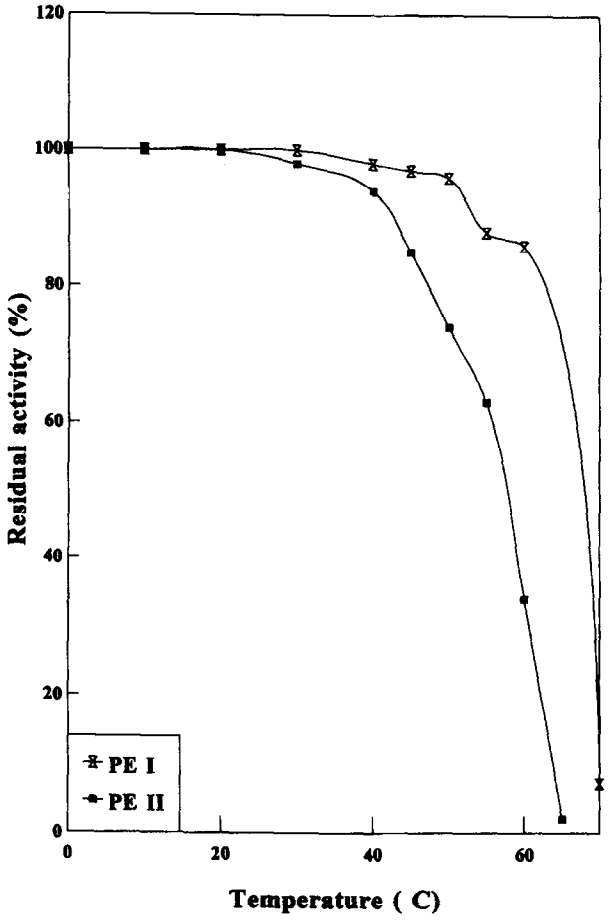

Fig. 1. Heat stability of PE I and PE II in 20 mM phosphate buffer pH 7.5. PE I and PE II were incubated in the phosphate buffer for 5 min at various temperatures and the residual activity was determined under standard assay conditions.

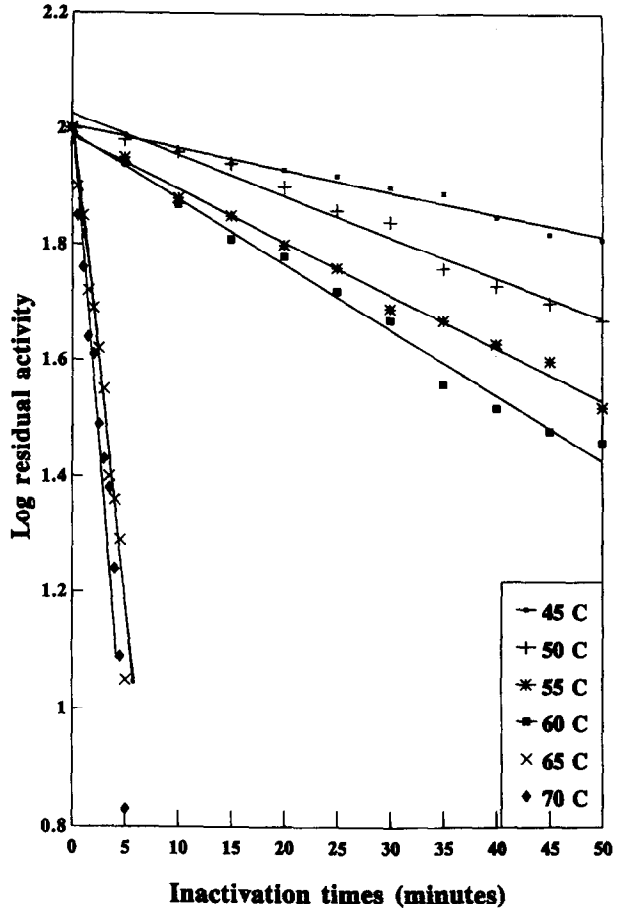

Fig. 2. Thermal inactivation of PE I in sodium phosphate buffer of pH 7.5. PE I was incubated in 11.5 ml of 20 mM sodium phosphate buffer for several time intervals at several temperatures as indicated in the figure. Residual activities were determined under standard assay conditions.

PE I, PE II and high molecular weight PE from orange juice were rapidly inactivated at 60, 70 and 90°C respectively. Heating for 5 min at 63 and 64°C gave 50% inactivation of PE A and PE B of tomato respectively, whereas the inactivation temperature for PE C was noticeably lower at 55°C (Warrilow *et al.*, 1994). Cameron *et al.* (1994) provided PE data from citrus fruit showing that PE 3 retained 29% activity after being incubated at 95°C for 30 s and the other two forms also retained activity at 80 and 90°C for the same time.

The heat inactivation rates of purified PE I and PE II at pH 7.5 are shown in Figs 2 and 3 respectively. The *D* values at 65°C were approximately 5.8 min and 3.3 min for PE I and PE II respectively. It shows that for 90% inactivation at 65°C, PE I required 2.4 min more heating time than PE II. However, the results reported here were for purified soursop pectinesterase in buffer at pH 7.5. In the fruit juice industry they generally employ high-temperature short-time (HTST) heat treatment and the pH of the juice is lower. According to Fayyaz *et al.* (1995b) the heating time and temperature required to inactivate pectinesterase decreased with pH. Versteeg *et al.* (1980) demonstrated that orange juice contained two isozymes of pectinesterase that were heat labile at 70°C and a third isozyme that was stable at temperatures up to 80°C. They also showed that cloud loss in orange juice was due to improper inactivation of the heat stable isozyme. Nath and Rangganna (1977) observed that at pH values of 3.6 and 4.0, the *D* and *F* values for pectinesterase of citrus juice were 1.0 min at 91.94°C and 93.61°C respectively. A *D* value of 7.42 min at pH 7.5 was estimated for papaya pectinesterase by Fayyaz *et al.* (1995b).

The changes in temperature required to increase the inactivation rate tenfold (*Z* value) were calculated at 8.5 and 8.6°C for PE I and PE II respectively (Fig. 4). These values were close to the *Z* values of purified PE obtained from other fruits. Wicker and Temelli (1988) calculated *Z* values for the heat sensitive and heat stable pectinesterase fractions in orange juice pulp

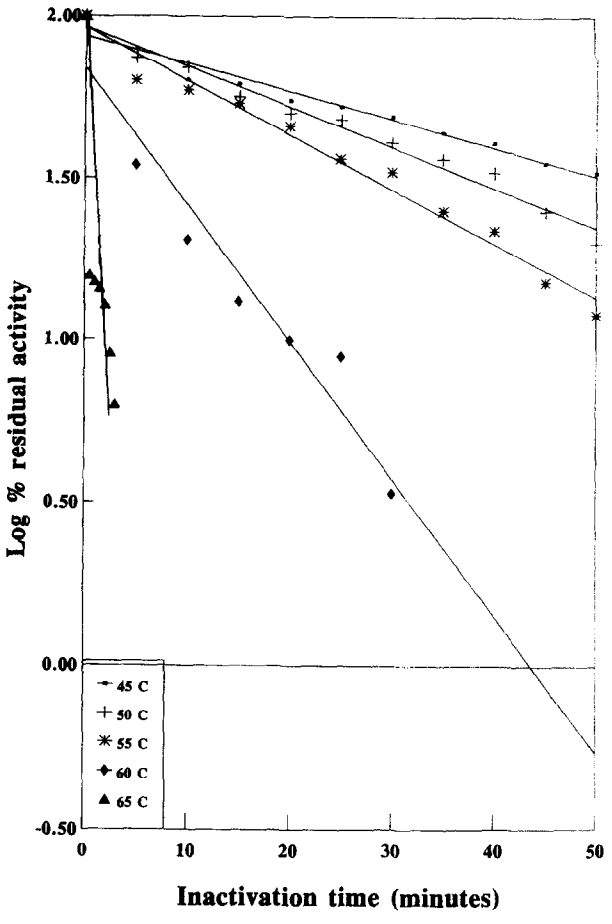

Fig. 3. Thermal inactivation of PE II in sodium phosphate buffer of pH 7.5. PE II was incubated in 11.7 ml of 20 mM sodium phosphate buffer for several time intervals at several temperatures as indicated in the figure. Residual activities were determined under standard assay conditions.

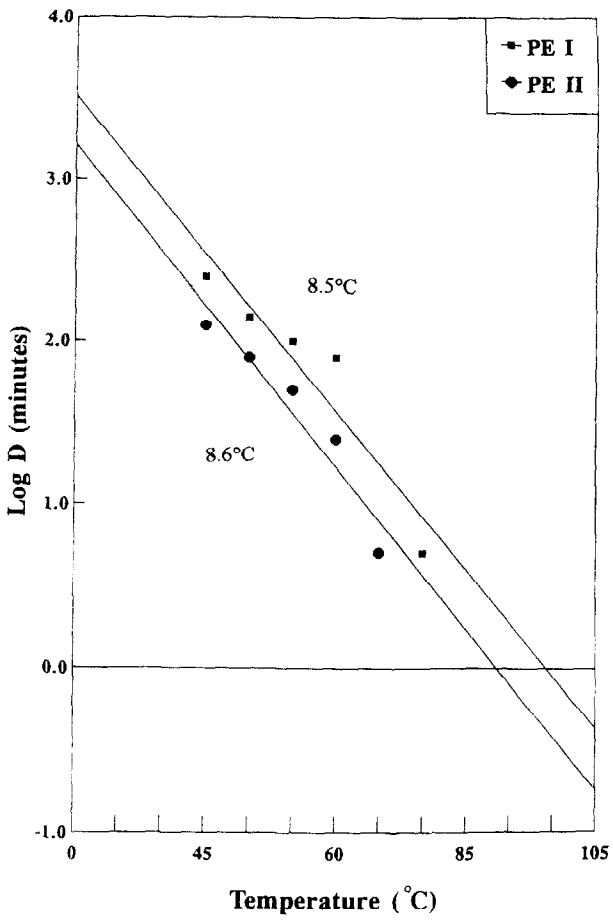

Fig. 4. Thermal destruction curves for PE I and PE II in 20 mM sodium phosphate buffer of pH 7.5.

which were estimated to be 10.8 and 6.5°C, respectively. Versteeg *et al.* (1980) reported Z values of 6.5°C for PE I and PE III and a Z value of 11°C for PE II. The Z values estimated by Fayyaz *et al.* (1995b) for papaya pectinesterase were 7.8 and 8.4°C at pH 7.5 and 4.0 respectively.

Effect of purified soursop PE on cloud stability of soursop juices

The plots in Figs 4 and 5 show cloud destabilisation in natural and heat treated juices at different storage temperatures. The cloud of heated juice remained quite stable compared to natural juices and heated juices in which purified PE I and PE II were added. Since the PE was not active in heat treated juices, the cloud loss was probably due to precipitation of cloud particles. Heat treatment may have caused some disintegration of the larger cloud particles. Furthermore, heat treatment might cause extraction of pectin from cloud particles into the serum, which would result in a new substrate for pectinesterase (Krop, 1974). Another possible explanation for this is the presence of residual active enzyme, which could contribute to the gradual loss of cloud which is observed in the storage conditions of soursop juices (MacDonald *et al.*, 1993).

Both enzymes were found to destabilise the cloud of soursop juices at pH 3.7. Natural juice clarified the fastest (large decrease in A 660 nm) at both storage temperatures. The cloud loss was greater for natural juice as compared to the heated juice with added purified PE. At 4°C it took longer time before clarification occurred because the activity of pectinesterases was low as compared to storage conditions at 30°C. Also Fig. 6 shows that for heated juice with added PE I cloud loss is greater than for PE II since PE I is more heat stable than PE II. This is in agreement with Versteeg *et al.* (1979), who observed that the high molecular weight pectinesterase (thermostable PE) quickly destabilised the cloud of orange juice that had been kept at 30°C. Clarification became apparent within 6 h whereas at 5°C juice storage the cloud was stable for 25 days.

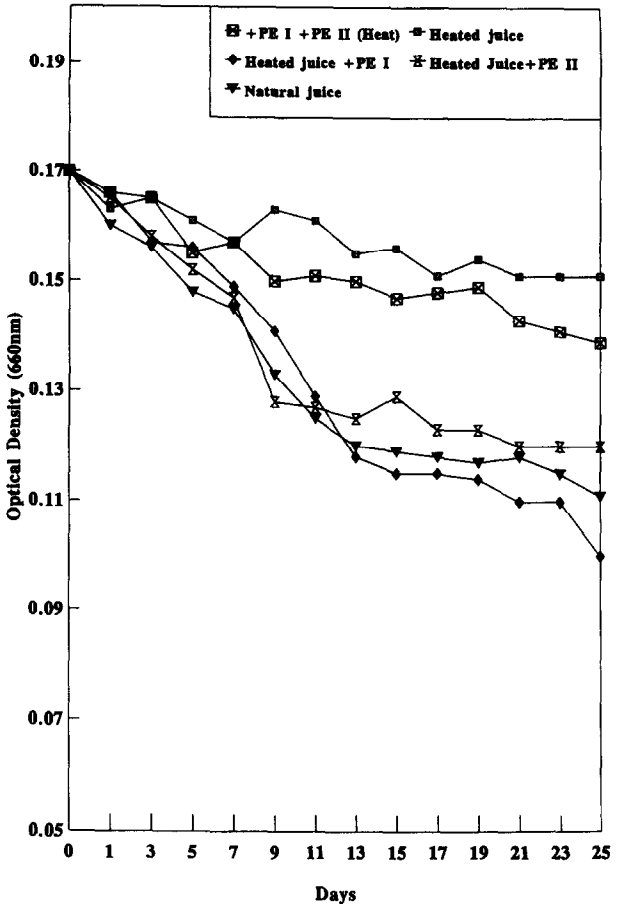

Fig. 5. Effect of purified soursop pectinesterases on cloud stability of soursop juice stored at 4°C storage temperature. Clarification was expressed as extinction of supernatant after centrifugation.

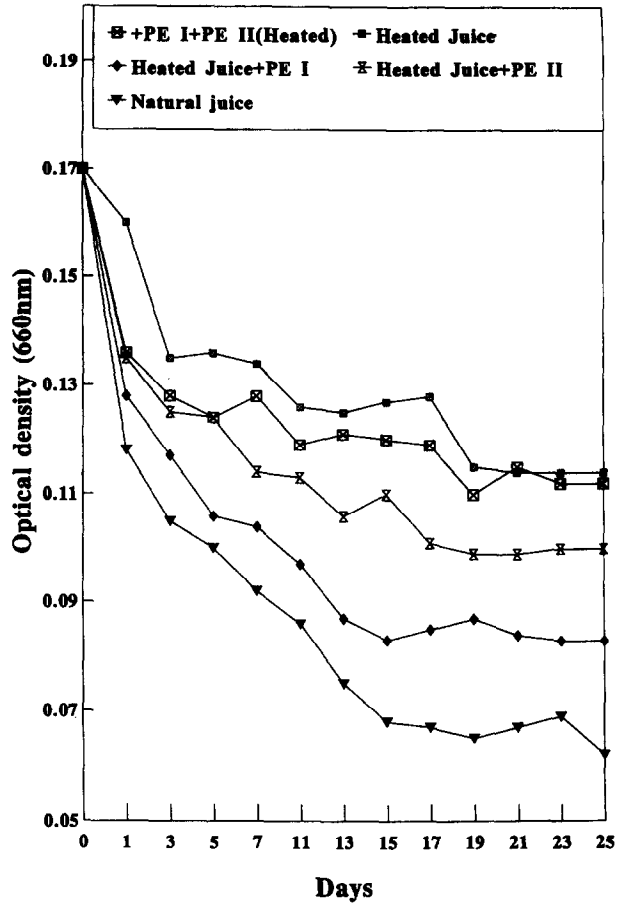

Fig. 6. Effect of purified soursop pectinesterases on cloud stability of soursop juice at 30°C storage temperature. Clarification was expressed as extinction of supernatant after centrifugation.

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