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# Partial purification, heat stability and kinetic characterization of the pectinmethylesterase from Brazilian guava, Paluma cultivars

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**Abstract**

Pectinmethylesterase (PME) was extracted from guava fruit (*Psidium guajava* L.), cultivar Paluma, by 70% ammonium sulphate saturation and partially purified by gel filtration on Sephadex G100. Gel filtration showed PME isoenzymes with different values of molecular mass. Two samples were examined: concPME (70% saturation by ammonium sulphate) and Iso4 PME (one of the isoforms from gel filtration with the greatest specific activity). Optimum pH of the enzyme (for both samples) was 8.5 and optimum temperature ranged from 75 and 85 °C. The optimum sodium chloride concentration was 0.15 M. The $K_M$ and $V_{max}$ ranged from 0.32 to 0.23 mg $ml^{-1}$ and 244 to 53.2 μmol/min, respectively, for concPME and Iso4PME. The activation energies ($E_a$) were 64.5 and 103 kJ/mol, respectively, for concPME and Iso4PME. Guava PME, cv Paluma, is a very thermostable enzyme, showing great heat stability at all temperatures studied.


*Keywords:* Pectinmethylesterase; Isoenzymes; Heat stability; Guava fruit

## 1. Introduction

Brazil with its 2.2 millions hectares of fruit cultivars, is one of the biggest fruit producer of the world, producing more than 30 millions tons annually, which is approximately 10% of the total world fruit production (Musser, 1995). The culture of guava is one of the principle activities of the horticulture of the State of São Paulo, and the production of the guava for export is of great economic importance (Martin & Kato, 1988; Pereira & Martinez, 1986).

The Paluma guava cultivar was obtained from the Ruby-supreme guava cultivar (Pereira & Martinez, 1986). It is very productive; it has late production, with large fruits (average weight 160 g), of oblong form, with pulp stable in transport and conservation, low number and medium size of seeds and pleasant flavour and odour (Pereira & Martinez, 1986). Guava fruit is rich in antioxidant activity, maybe due to its high vitamin C content (the concentration is ten times higher than in orange) (Ito, Yamaguchi, Ohata, & Ishihata, 1980), as well as sugar, vitamins A and B, pectic substances (pectin), proteins and mineral salts, mainly iron, calcium and phosphorus. It is consumed fresh or made into juice, nectar, puree, jam or jelly. Guava has been used as an additive in other juices and purees, thus increasing the content of vitamin C (Martin & Kato, 1988; Medina, 1988). Due to its good pectin content, it has viscosity properties useful for obtaining purees. Guava puree is reprocessed into nectars, juice drink blends, jams, jellies

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and syrups that give it great industrial application (Chem Chin et al., 1984, Ito et al., 1980; Kashyap, Vohra, Chopra, & Tewari, 2001; Seymour, Taylor, & Tucker, 1993).

The enzyme pectinmethylesterase (PME; EC: 3.1.1.11), also known as pectin esterase, catalyses hydrolysis of the methoxyl group of pectin, forming pectic acid as a product of the reaction. This enzyme acts preferentially on a methyl ester group of a galacturonate unit next to a non-esterified galacturonate unit. The decrease in the degree of pectin methoxylation, may in its turn, trigger different processes related to texture and firmness (Tijskens, Rodis, Hertog, Proxenia, & Van Dijk, 1999). Such catalytic action makes PME one of the most important enzyme in the industrialization and preservation of fruits, juices or other industrial products that involve the presence or absence of intact pectin (Alonso, Howell, & Canet, 1997; Giovane, Quagliolo, Servillo, & Balestrieri, 1990; Javeri & Wicker, 1991; Lin, Liu, Chen, & Wang, 1989; Nighojkar, Srivastava, & Kumar, 1995). PME commonly occurs in various parts of higher plants, including fruits, and it has been found in pathogenic fungi and bacteria (Arbaisah, Asbi, Junainah, & Jamilah, 1996; Giovane et al., 1994). Studies of PME in fruits show that it normally exists as two or more isoforms, each comprising a simple polypeptide chain with molecular mass ranging from 10 to 60 kDa (Alonso et al., 1997; Giovane et al., 1990; Markovic & Joernvall, 1992).

Previous research on pectinmethylesterase carried out in our laboratory, with samples provided by food industries of the Araraquara region, has shown residual enzyme activity at different steps of the industrial processing of guava fruit. The samples analysed included fresh fruit upto the production of guava sweet, and also stored guava puree (no published result). Those studies suggested that residual catalytic PME action is probably responsible for the necessity of adding commercial pectin to stored guava pulp during industrial processing, causing increase of the cost of the final product. The purpose of this work was to study the guava PME enzyme, by kinetic characterization, in order to check the presence of isoenzymes and to determine PME heat stability, which is important for the food industry of the Araraquara and Monte Alto regions, State of São Paulo, Brasil.

## 2. Materials and methods

### 2.1. Fruit sampling and preparation of the pulp

Guava fruits, Paluma cultivars, were collected from several points of the boxes containing fruits awaiting industrial processing. To assure a representative sample, the use of this variety was suggested by Indústria e Comércio de Conservas Alimentícias PREDILETA-Ltda., de São Lourenço do Turvo, Matão-SP, Brasil, since it is the one most used (90% of the total of the processed fruits) in the industrial processing of guava products (sweet, gels, purees and others). The fruits were kept frozen (below 4 °C).

### 2.2. Extraction of the PME

The pulp was obtained by passing the fruits through a despulper.

Unless otherwise indicated, all steps were performed at 4 °C. The pulp was homogenized in a blender with borate–acetate (extractor buffer), 50 mM, pH 8.3, containing 0.20 M NaCl. The ratio of the pulp mass (g) to buffer solution (ml) was 1:3.The mixture was centrifuged at 15,000*g* for 10 min, and the supernatant was then squeezed through cheese cloth to remove solid particles (Korner, Zimmermann, & Berk, 1980). Then, filtrate supernatant was brought to 70% saturation by addition of solid ammonium sulphate, and was centrifuged at 15,000*g* for 10 min after standing for 2 h. The pellet was suspended in cold borate–acetate buffer in a ratio of 1:1 (w/v). This fraction was named *concentrated PME (concPME)*.

### 2.3. Partial purification of concPME

Two millilitres of the concentrated PME sample (2.25 mg protein/ml) were loaded in a Sephadex G100 column (from Sigma Chemical Co), column size $48 \times 1.2$ cm, previously equilibrated with borate–acetate buffer, pH 8.3, with 0.15 M NaCl. Elution was performed at a flow rate of 0.25 ml/min, and fractions of 2 ml were collected. All fractions with absorbance at 280 nm were assayed for PME activity, pooled and stored at −15 °C. This pooled enzyme sample was named Iso4 PME.

### 2.4. PME activity assay and protein determination

The PME activity was determined by measuring the amount of free carboxyl groups formed as a result of enzyme action on pectin (as substrate) by the titrimetric method of Kertesz (1955). The standard reaction mixture consisted of 0.125% citrus pectin solution (substrate, 29.5 ml), 0.15 M NaCl and enzyme solution (0.5 ml). After the pH was adjusted to 8.0 with 0.01 M NaOH or 0.01 M HCl, this reaction mixture was incubated at 50 °C and titrated with 0.1 M NaOH. Units of PME (UA) and specific activity were calculated according to Assis, Lima, and Oliveira (2000).

### 2.5. Protein determination

Protein concentration was determined in all enzyme extracts from the fruits by Hartree (1972) method, using

bovine serum albumin (Sigma Chemical Co.) as the standard for calibration. The eluted fractions from the chromatographic separation was also monitored at 280 nm in a UV/VIS diode array spectrophotometer, Model HP 8452A coupled to a chemstation.

### 2.6. pH optimum determination

The pH dependence of the PME enzyme activity was examined within the range pH 7–10. Activity at each pH value was determined by the standard enzyme assay (PME units/ml) and expressed as specific activity (PME units/ml/mg protein). Control assay of pectin without enzyme PME was carried out for each pH, and named pectin blank. The amount of acid produced, due to the spontaneous pectin demethylation by pH, was subtracted from the values obtained in the corresponding systems where PME enzyme was present.

### 2.7. Temperature optimum determination

The effect of temperature on PME activity (UA) was determined by measuring the enzyme activity between 35 and 90 °C at optimum pH, and expressed as specific activity. Control assays of pectin without PME were done at each temperature, and the amount of acid produced by the spontaneous pectin demethylation, due to temperature, was subtracted from the values obtained in the corresponding systems where PME was present (Fig. 1).

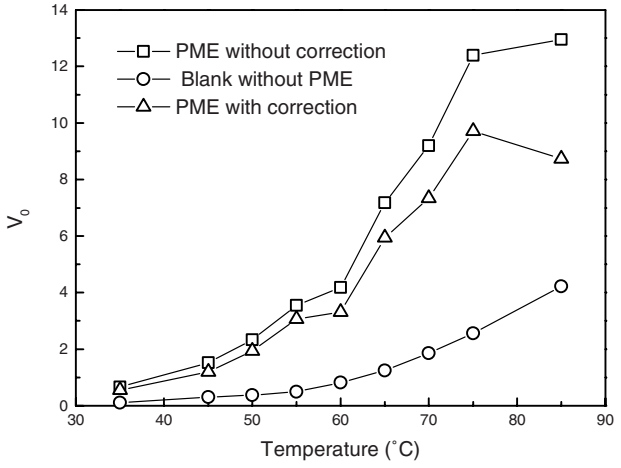


Fig. 1. Investigation of the effect of temperature on the pectin stability. This experiment was done to examined by plot of initial rate of the reaction ($v_0$) versus temperature the effect to pectin molecule (spontaneous pectin de-sterification). It was also done for pH and NaCl experiments. ConcPME without correction (□) kinetic assay: 0.5 ml PME solution, 0.125% citrus pectin, 0.15 M NaCl and pH equal 8.0. Blank without PME (○) kinetic assay: 0.5 ml buffer borate–acetate, 0.125% citrus pectin, 0.15 M NaCl and pH equal 8.0. ConcPME curve with correction as Δ, it is equal (□) minus (○) ($n = 3$).

### 2.8. Heat stability determination

Aliquots of concPME or of Iso4 PME in test tubes (selected to be equal in weight and size) were incubated in a water or glycerine bath, at different temperatures, and for various periods of storage. After the heating process, the tubes were cooled in melting ice for 30 min. The residual PME activity was measured to verify the effect of temperature during storage. The concPME was incubated for various times at the following temperatures: 50, 75, 90 and 98 °C (1, 10, 20, 30, 40, 50 and 60 min); 90 °C (3, 4, 5, 6, 7 and 8 h); 106, 112 and 125 °C (5 min). The Iso4 PME was incubated at 75 °C (1, 10, 20, 30, 40, 50 and 60 min) and at 90 and 98 °C (20 and 40 min).

### 2.9. Effect of monovalent cations

The effect of $Na^+$ ions on concPME activity was examined at NaCl concentrations of 100–600 mM, and the Iso4 PME was examined in NaCl (100–300 mM). Also, the concPME activity was examined in the presence of 150 mM NaCl, LiCl, KCl and RbCl, to investigate the best monovalent cations for this PME activation.

### 2.10. Kinetic parameters of PME

The initial reaction rate ($v_0$) in the presence of various substrate concentrations (citric pectin, from Sigma Chemical Co.) was determined. The $K_M$ and $V_{max}$ of the PME were determined from a Lineweaver–Burk plot with citrus pectin as the substrate at optimum pH, NaCl concentration and temperature for each particular PME guava extract.

### 2.11. Molecular mass determination

The molecular mass of guava PME was estimated by gel filtration on Sephadex G100, according to the method of Whitaker (1963) and 2.0 ml per fractions were collected. The mixture of bovine serum albumin (66,000 Da), carbonic anhydrase (29,000 Da), cytochrome *c* (13,400 Da) and tannic acid (1852 Da), dissolved in borate–acetate buffer and applied to the column, was used for molecular mass calibration G100 column. The void volume was determined by Blue Dextran 2000 solution.

### 2.12. Statistical analysis

Average values of triplicates were calculated. The data obtained from the studies were analysed using linear or quadratic regression.

### 2.13. Determination of the Arrhenius activation energy

The activation energy, $E_a$ was estimated for concentrated and Iso4 PME by using the Arrhenius equation

$k = Ae^{-E_a/RT}$,

where $k$ is the rate constant of the reaction at the temperature $T$ (in Kelvin degrees); $A$ is a pre-exponential factor (number of the shocks per second); $E_a$ is the activation energy; $R$ is universal gas constant (equal at 8.3145 J $K^{-1}$ $mol^{-1}$) and $e^{-E_a/RT}$ is the fraction of the molecules of the system at a particular temperature $T$ that has the measured energy $E_a$ (Boltzman factor).

The activation energy ($E_a$) was calculated from the slope of the linear plot ln $k$ versus $1/T$ ($K^{-1}$).

## 3. Results and discussions

### 3.1. Partial purification

The data from loaded samples (concPME) on the Sephadex G100 column showed the presence of PME isoenzymes (Fig. 2), and the highest specific activity was peak four and it was called Iso4 PME. Table 1 shows a 104-fold purification of PME enzyme, compared to the values obtained for the crude extract and the concPME.

### 3.2. Optimum pH

It was verified that the optimum pH of the concPME occurs between the values 8.0 and 9.0 (Fig. 3). PME Iso4

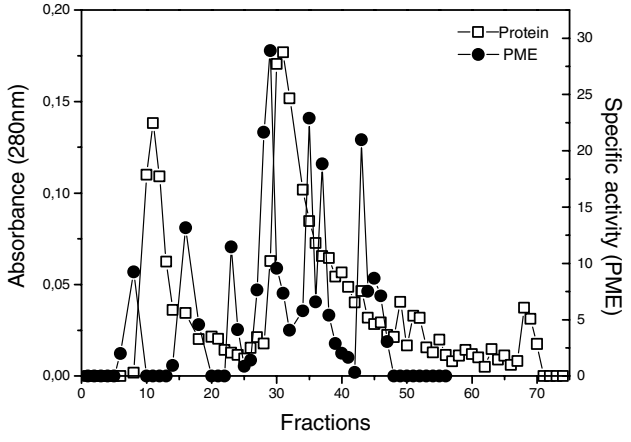


Fig. 2. Elution of the concPME solution on Sephadex G100 column. Kinetic assay: 0.5 ml PME, 0.125% citrus pectin, 0.15 M NaCl, 50 °C temperature and pH equal 8.0. Protein content is shown as □, and PME activity is shown as ● ($n = 3$).

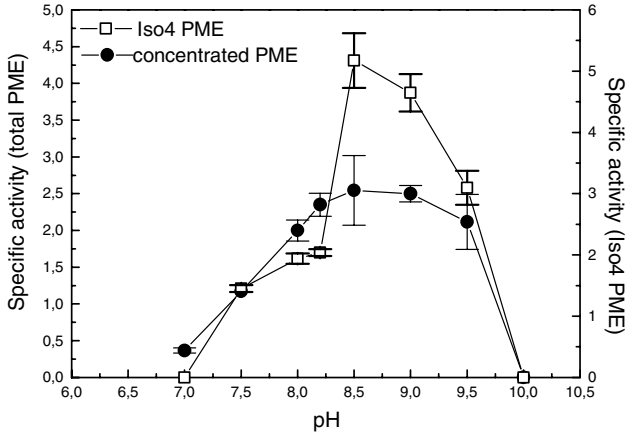


Fig. 3. Optimum pH determination of the concPME and Iso4PME activity. All reactions were carried out at pH range 7.0–10.0, with 0.125% citric pectin, 0.15 M NaCl, 0.5 ml PME, temperature 50 °C. Total PME activity is shown as ●, and Iso4 PME activity is shown as □ ($n = 3$).

also showed optimum pH at 8.5. Optimum pH varies for fruits; in general, PME has been found to have an optimum pH from 7.5 to 9.0 (Arbaisah, Asbi, Junainah, & Jamilah, 1997; Assis et al., 2000; Lim & Chung, 1997). Orange PME, from differents cultivars, also has been found to have an equal range of optimum pH (Amaral, Lima, & Oliveira, 1993; Cameron & Grohmann, 1996; Termote, Rombouts, & Pilnik, 1977). The pH importance for action of the PME has been related to the defensive (or protector) effect at pH values near to 8.0 (Atkins & Rouse, 1953; Eargeman and Rauganna, 1997; Moustacas, Nari, Borel, Noat, & Ricard, 1991).

### 3.3. Optimum temperature

The data showed optimum temperatures at 75 and 85 °C for the concPME and Iso4 PME samples, respectively (Fig. 4). The results obtained agree with studies of PME from different vegetable sources, which showed optimum temperature values within the range 50–90 °C (Assis, Martins, Guaglianoni, & Oliveira, 2002; Korner et al., 1980), and are higher than those data obtained with microbial PME, that ranged 30–70 °C (Kashyap et al., 2001). All determinations of the PME activity in the present work were carried out at 50 °C, due to the pectin instability at high temperature and where the control pectin assay showed a smaller effect.

Table 1
PME activity for the various steps of enzyme purification. Enzyme activity is expressed as μmol of released carboxyl groups per minute at 50 °C[a]

| Purification step | Total activity | | | |
|---|---|---|---|---|
| | Protein/mg $ml^{-1}$ | (U)/μmol $min^{-1}$ ml | Specific activity/μmol $min^{-1}$ $mg^{-1}$ | Purification (fold) |
| Crude extract | 1121 | 172 | 0.15 | 1 |
| Ammonium sulphate fractionation (70%) | 56.2 | 178 | 2.09 | 13.9 |
| Iso4 PME (from gel Sephadex G100) | 9 | 136 | 15 | 104 |

[a] Enzyme assays and protein determinations were performed as described in Section 2. Results are means of at least three determinations.

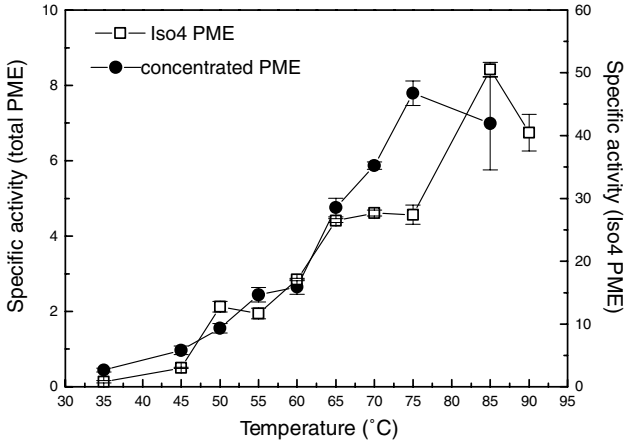


Fig. 4. Effect of temperature on concPME and on Iso4 PME activity. All reactions were carried out at pH 8.0, with 0.125% citric pectin, 0.15 M NaCl, 0.5 ml PME, and at different temperatures from 35 to 90 °C. Total PME activity is shown as ●, and Iso4 PME activity is shown as □ ($n = 3$).

### 3.4. Effect of cations on the catalytic action of PME

From Fig. 5, it can be seen that 0.10 to 0.20 M NaCl stimulates the PME. Optimum concentrations of NaCl were 0.20 and 0.15 M, respectively, for concPME (specific activity = 9.87 units/mg of protein) and for Iso4-PME (specific activity = 15.67 units/mg of protein). On further increase of $Na^+$ ion concentration, the enzyme activity decreased. $Na^+$ ions are believed to bind to the enzyme, inducing conformational modification, favouring reaction of the enzyme with substrate (Nari, Noat, & Ricard, 1991).

As shown in Table 2, concPME also had high activity with $Li^+$, $K^+$ and $Rb^+$ monovalents ions at 0.15 M, but with slightly lower values than with $Na^+$ ions. These results are in agreement with Assis et al. (2002), for PME from acerola in the presence of the $Na^+$, $K^+$, $Li^+$, $Ca^{2+}$ and $Mg^{2+}$ who showed that $Na^+$ was the best choice and led to a higher catalytic action of PME. Nari et al. (1991) suggested that the PME activation by cations could be due to interaction with carboxyl groups present in the pectin molecule.

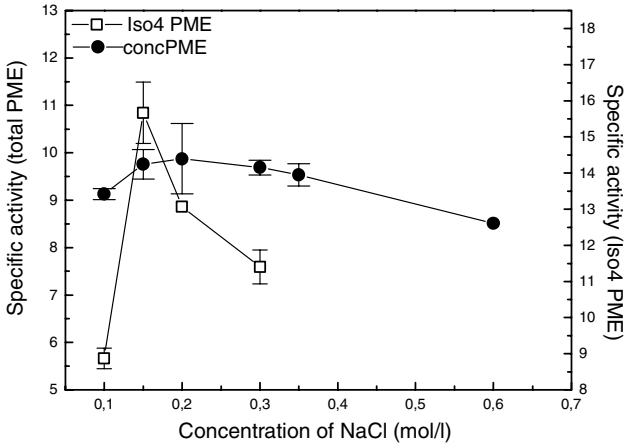


Fig. 5. Effect of concentrations of NaCl on the guava PME activity. ConcPME is shown as ● and Iso4 PME as □. All reactions were carried out at pH 8.0, with 0.125% citric pectin, 0.5 ml enzyme, on temperature 50 °C and at various concentrations of NaCl from 0.10 to 0.60 M ($n = 3$).

Table 2
Effect of monovalent cations on the activity of the concPME from guava cv Paluma

| Monovalent cations | Enzyme activity (μmol/min/mg) |
|---|---|
| Without cations | 3.13 ± 0.04 |
| $Li^+$ | 3.29 ± 0.04 |
| $Na^+$ | 3.83 ± 0.16 |
| $K^+$ | 3.22 ± 0.20 |
| $Rb^+$ | 3.24 ± 0.14 |

All reactions were carried out at pH 8.0, with 0.125% citric pectin, 0.5 ml PME solution, temperature 50 °C and in 0.15 M salt solutions: LiCl; NaCl; KCl and RbCl.
Results are means of at least three determinations.

Studies carried out by Moustacas et al. (1991), with PME from different sources, suggested a relationship between pH of the reaction mixture and salt concentration: (i) at high value of pH (i.e., pH 8.0), the maximum PME activity was observed at low salt concentrations and, (ii) at low pH, there was observed high PME activity at high salt concentrations. Our results are in agreement with the previous suggestion. Moustacas et al. (1991) also suggested that the PME, the pH and cation concentration, all have important roles in the growing cell wall.

### 3.5. Determination of the activation energy $E_a$

From the linear plots of ln $k$ versus $1/T$ ($K^{-1}$), values of $E_a$ equal to 64.5 and 103 kJ/mol were obtained for concPME and Iso4PME, respectively. These values are considerably higher than those obtained for other PME sources, such as: graviola pulp (*Anona muricata*), in which $E_a$ ranged from 36.0 to 42.0 kJ/mol (Arbaisah et al., 1997), peaches with $E_a$ of 34.6 kJ/mol (Javeri & Wicker, 1991), and PME isoforms from orange pulp with $E_a$ values of 23.4 and 24.0 kJ/mol (Korner et al., 1980). As a consequence, this enzyme, *Paluma* guava PME, has a low reaction velocity in the optimum conditions assay, and then it shows great sensitivity in relation to change of temperature.

### 3.6. Determination of the kinetic constant $K_M$ and $V_{max}$

Table 3 shows the Lineweaver–Burk plot used to calculate $K_M$ and $V_{max}$ of the concPME and Iso4PME, as well the relationship between our results and those from others sources. The $K_M$ values of PME varied from 0.23 to 0.32 mg ml$^{-1}$, values comparable to the $K_M$ of fruit PME, which was from 0.083 to 0.52 mg ml$^{-1}$ (Alonso

Table 3
The relationship between our results and values obtained from other sources

| PME Enzyme source | $K_M$ (mg ml$^{-1}$) | $V_{max}$ (μmol ml$^{-1}$ min$^{-1}$) | Reference |
|---|---|---|---|
| Concentrated from guava | 0.32 | 0.244 | This work |
| Iso 4 from guava | 0.23 | 0.053 | This work |
| Isoenzyme from persimmon | 0.0010–0.075 | | Alonso et al. (1997) |
| Isoenzyme from string beans | 0.104–0.049 | | Laats et al. (1997) |
| Isoenzyme from graviola pulp | 0.52–0.083 | | Arbaisah et al. (1997) |

et al., 1997; Arbaisah et al., 1997; Assis et al., 2002; Lim & Chung, 1997; Macdonald, Evans, & Spencer, 1997). The ratio $K_M/V_{max}$, which corresponds to the catalytic velocity constant $k_{catal}$ varied from 0.05 to 0.17 min$^{-1}$. This suggests that guava PME can occur as an enzyme complex, or in association with another biomolecule, that hinders and/or regulates its catalytic action. Our results are in agreement with others obtained from different sources of PME.

### 3.7. Heat stability of PME

Heat stability of the guava concPME activity, as a function of time (minutes) of incubation at various temperatures (from 50 to 125 °C), is shown in Fig. 6. The PME is very stable, showing activity even after 30 min of storage, independently of the temperature studied. At 75 °C, there is an increase of the specific activity after 30 min of storage. This behaviour is possibly due to liberation of the enzyme molecule from complex structure or from regulators/inhibitors present in the enzyme sample.

Elution of concPME solution on a Sephadex G100 column, showed a profile of isoforms, with estimated molecular masses ranging from 43,916 to 0.75 kDa. These results, coupled with the thermostability at 75 °C, lead us to believe that the PME would be present in aggregate form with its subunits forming a great complex and/or associated with another biomolecule, conferring stability to the aggregate. Probably, the heating process liberates PME from the aggregate, giving an increase of the specific activity, as observed at 75 °C. When the Iso4 PME was studied at 75 °C, similar behaviour was observed, but with low intensity. Fig. 7 shows the high thermostability of the Iso4 PME and concPME on temperatures from 75 to 98 °C. Considering the high thermostability of this PME (45 min at 98 °C without significant loss of its activity), the enzyme was investigated at 106, 112 and 125, stored for 5 min, and Fig. 8 illustrates the residual activity (%) after storage at those temperatures. At 125 °C, the loss is only 20%.

The high thermal stability of guava PME, cv Paluma, makes it possible that its catalytic action continues during the pulp storage period. As a consequence, there is a

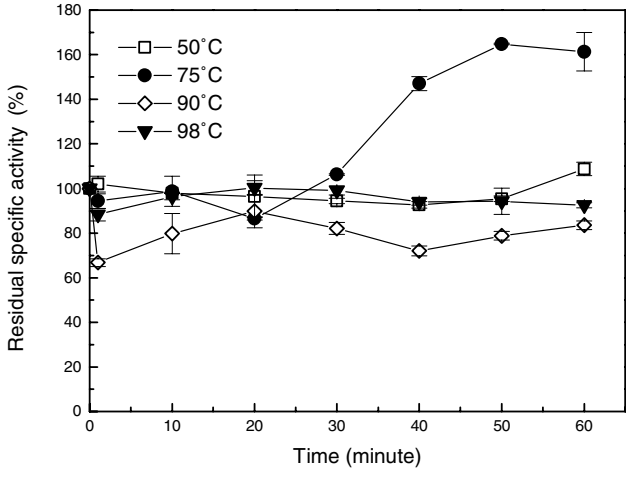


Fig. 6. Heat stability of guava concPME activity as a function of time (minutes) of the incubation on different temperatures. Storage on temperature at 50 °C is shown as □, at 75 °C as ●, at 90 °C as ◇, at 98 °C as ▼. All reactions were carried out at pH 8.0, with 0.125% citric pectin, 0.15 M NaCl, 0.5 ml PME solution, and at temperature 50 °C (after storage at cited temperatures in different times) ($n = 3$).

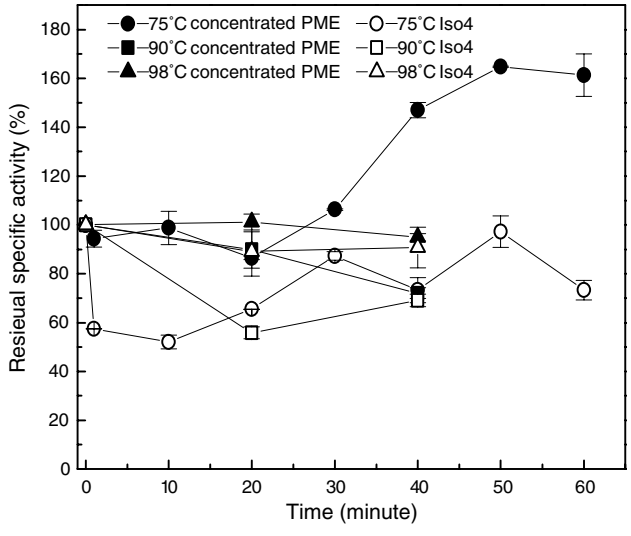


Fig. 7. Heat stability of guava PME activity solution, as a function of time of incubation on different temperatures. Storage on temperature at 75 °C is shown as ● and ○; at 90 °C as ■ and □; at 98 °C as ▲ and Δ, respectively. Further all enzymatic reactions were carried out at pH 8.0, with 0.125% citric pectin, 0.15 M NaCl, 0.5 ml enzyme, and at temperature 50 °C (after storage at cited temperatures in different times) ($n = 3$).

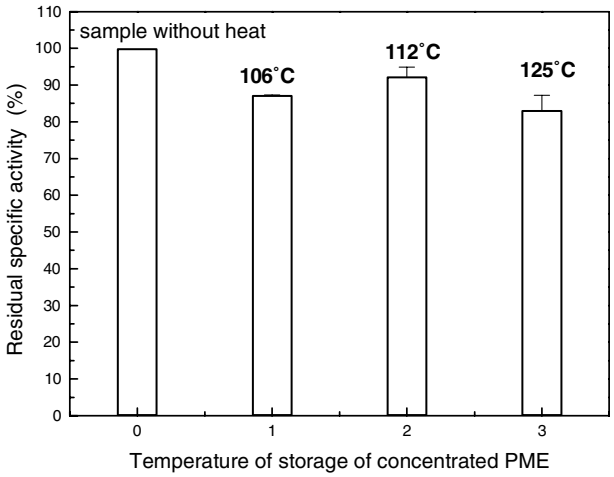


Fig. 8. Heat stability of concPME activity at: 106 °C (number 1), 112 °C (number 2) and 125 °C (number 3) for 5 min of the storage incubation. Sample enzyme without heat (control) is number 0. Further all enzymatic reactions were carried out at pH value 8.0, 0.15 M NaCl, with 0.125% citric pectin and at temperature 50 °C (after storage on cited temperatures for 5 min and cooled) ($n = 3$).

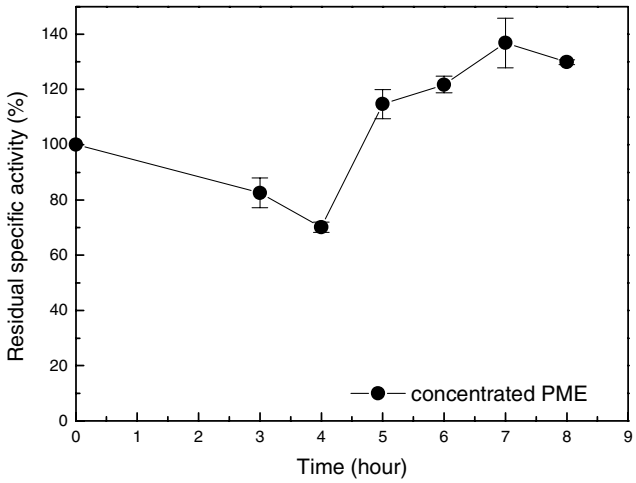


Fig. 9. Heat stability of concPME activity as a function of time (hours) of incubation at 90 °C. Further all enzymatic reactions were carried out at pH value 8.0, 0.15 M NaCl, with 0.125% citric pectin and at temperature 50 °C (after storage on cited temperature and cooled) ($n = 3$).

loss of pectin with the degree of esterification needed (proper consistency) for guava sweet production.

Assis et al. (2000) studying acerola PME, reported a loss of about 90% of PME activity in 2 min of incubation at 106 °C. Cameron and Grohmann (1996) studying citric juice isoenzymes, observed that one of the isoforms was more thermostable, retaining 49.2% of its initial activity after 1 min of incubation at 95 °C (loss of 50.8% of activity). Javeri and Wicker (1991) reported that peach PME loses 77% of its activity when incubated for 5 min at 65 °C, and is completely inactivated at 70 °C for 5 min. Seymour et al. (1991) isolated two PME isoenzymes from grapefruit and verified that they differed in relation to the amount of associated carbohydrates. With the removal of these carbohydrates, there was a decrease of the thermostability, suggesting that these molecules contributed to the thermal stability of the PME.

Study of PME from five cultivars of tomato (Larata et al., 1995) showed that one of them had high thermal stability, and this could explain the loss of consistency of some industrial products of tomato during storage. Macdonald et al. (1997) using continuous flow assay, isolated four isoforms of lemon PME, one of them showing high thermal stability, retaining its activity after being stored at 86 °C for 9 min. The authors ascribed the destabilisation of the lemon juice to the thermostability of this isoforms.

The study of the effect of temperature on stability and catalytic action of enzymes as PME is of great importance for the food, juice and fruit sweets industries, mainly due to the decrease of the degree of the esterification of pectin molecules. This decrease is responsible for the difficultly of reaching the final point in the production of sweets, or in maintaining juice stability, which can lead to quality loss of the final product. In order to compensate for this loss, the addition of commercial pectin is necessary, which increases the costs of production. So, it is desirable that this enzyme be inactivated during industrial processing.

The food industry generally treats the pulp at 90 °C for 1 min, aiming for inactivation of the PME enzyme. The present work showed that this treatment is ineffective. Fig. 9 illustrates the heat stability of the concPME when stored at 90 °C, during 8 h. Therefore, our work suggests that thermal treatment can be made at temperatures below 90 °C for 1 min, which should decrease the industrial costs and loss of nutrients.

## 4. Conclusions

The guava fruit analysed in the study showed significant PME activity and high thermostability. The results obtained explain, by themselves, why the industry has to add more pectin in order to obtain guava sweet from stored pulp. Studies to purify the PME extracted from guava fruits, cv Paluma, will help characterize the PME further, for it to be used in the food industry.

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