

# Thermal inactivation kinetics of partially purified mango pectin methylesterase

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## Abstract

Kinetic parameters of thermal inactivation of pectin methylesterase (PME) in a partially purified mango enzyme extract were determined. The PME of mango partially purified by salting out showed different patterns of thermal inactivation, indicating the presence of a thermostable fraction at 70 °C and a thermolabile fraction at lower temperatures. The inactivation of the thermostable fraction exhibited a linear behavior that yielded a z-value of 9.44 °C and an activation energy ( $E_a$ ) of 245.6 kJ mol<sup>-1</sup> K<sup>-1</sup> using the Arrhenius model. The thermostable mango PME fraction represented 17% of total crude enzyme extract, which emphasizes the importance of residual enzyme activity after heat treatment.

**Keywords:** thermal inactivation; pectin methylesterase; Ataulfo mango.

**Practical application:** In literature there is a lack of information about kinetic parameters describing the inactivation of pectin methylesterase in mango due mainly to the little or no activity of this enzyme in mango. This paper reports the kinetic of inactivation calculated from treatments at several times and temperatures. The information reported can serve as a reference when designing or applying heat treatments during pasteurization of fruit products.

## 1 Introduction

Turbidity and loss of consistency in fruit drinks are a common consequence of the activity of some endogenous enzymes or presence of microorganisms. This phenomenon can be a problem in processed product preservation (Castaldo et al., 1997). Within the enzyme systems present in the products obtained from fruit, is pectin methylesterase (PME, EC. 3.1.1.11), which belongs to the pectinesterase group. This enzyme catalyzes the hydrolysis of esterified groups of pectic substances, releasing methanol and forming precipitates by pectic acid interaction with calcium ions (Duvetter et al., 2009; Pilnik & Voragen, 1991; Vercet et al., 1999; Whitaker, 1993). Therefore, it is necessary that PME is inactivated or inhibited properly to stabilize or maintain the turbidity of certain processed fruit products (Espachs-Barroso et al., 2006; Vercet et al., 1999). On the other hand, this enzyme is one of the most common used to determine the efficiency of fruit and vegetables blanching (Yemenicioglu & Cemeroglu, 1988).

For fruit drinks and low-acid foods, thermal inactivation of enzymes involves the use of temperatures above 80 °C (Ramesh, 2007) in heat pasteurization processes (Vercet et al., 1999). However, PME enzymes have been found that exhibit a wide thermal stability range in orange, acerola and carrot juices. This suggests the presence of thermostable and thermolabile fractions of PME isoenzyme complexes with irregular characteristics (Arbaisah et al., 1996; de Assis et al., 2000; Ly-Nguyen et al., 2003; Versteeg et al., 1980). In this regard, it should be noted that the technological problems in citrus juice associated with PME have been attributed

to a high molecular weight thermostable fraction, which is also crucial in the establishment of pasteurization values in orange juice (Versteeg et al., 1980).

Several studies have reported a low enzyme activity in mango PME when compared to citrus and other fruits (Banjongsinsiri et al., 2004; Duvetter et al., 2009; Jamsazzadeh Kermani et al., 2015). Likewise, PME with different activities or characteristics has been reported in mango skin and pulp (Labib et al., 1995; Lizada, 1993). Additionally, the detection of mango PME inhibitors contributed to low levels of observed activity (Jamsazzadeh Kermani et al., 2015). Also, in studies with mango PME, kinetic inactivation parameters have been difficult to determine. In a study involving the analysis of Zebda variety mangos, PME thermal inactivation curves with irregular patterns were observed (Labib et al., 1995). Therefore, the objective of this study was to determine the kinetic parameters for thermal inactivation of PME from Ataulfo variety mango.

## 2 Materials and methods

### 2.1 Materials

Ripe Ataulfo mangoes in batches of 1.5 kg (17 °Bx, pH 4.1 ± 0.2) were purchased in the local market at the end of spring. Apple pectin (degree of esterification 70-75%) was obtained from Sigma-Aldrich and all used reagents were analytical grade.

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## 2.2 PME extraction

The puree prepared from mango pulp was obtained using a commercial juicer (Jack Lalanne Power Juicer, USA) and was subsequently filtered using cheesecloth and the retained portion (bagasse) was used for PME extraction following the methodology described by Vercet et al. (1999) with some modifications. For PME extraction, mango bagasse was suspended in a mixture of borate acetate buffer (B-A) with a pH of 8.3 in a proportion of 1:3, (0.45 mol/L boric acid, 0.1 mol/L sodium tetraborate, 0.3 mol/L sodium acetate and 0.2 mol/L NaCl). The mixture was stirred for 4 h followed by a 12 h resting period at 4 °C. The obtained extract was centrifuged at 3000 G and the supernatant was dialyzed for 12 h using BA diluted 1:1000 to prevent further flocculation in the salting out. Subsequently, the supernatant was subjected to salting out by differential precipitation with ammonium sulfate at 30-80% saturation. The precipitate was then dissolved again using a 1:3 proportion in 1:100 BA solution containing 0.1 mol/L NaCl and then dialyzed using a BA solution with a concentration of 1:1000. The resulting dialysate containing PME activity was considered pectin methylesterase crude extract (E-PME). The concentration of PMEs in fruits generally increases with maturity. In the case of mango, it has been reported that the PME activity increases as the fruit reaches 50% of its edible maturity and then decreases (Lizada, 1993). However in this study, the enzymatic activity of mango PME corresponded to a level five maturity with 15 °Bx (Kader, 2015). Mangos at this level of maturity were used because this is the stage in which the fruit is commercialized.

## 2.3 Determination of PME activity

The PME activity was quantified by titration of the carboxyl groups liberated from the pectin using an automatic pH-stat (Metrohm, Herisau, Switzerland). The quantification was carried out in triplicate. Modifications were made according to the method described by Kertesz (1955). The reaction mixture consisted of 10 ml of a solution of apple pectin at 1% as substrate, 0.2 mol/L NaCl, and 0.1 mol/L NaOH was used in order to adjust the pH to 8, taking into consideration the autodegradation of the pectin (Díaz et al., 2007; Renard & Thibault, 1996). In addition to this 0.3 mL of E-PME was added, reducing the pH to 7.5. The pH was maintained at 7.5 by the addition of 0.01 mol/L NaOH for 10 min at 30 °C. One unit of PME activity (UPME) was defined as the quantity of enzyme capable of releasing 1 mmol of carboxyl groups / min (Balaban et al., 1991) and calculated according to Equation 1 (de Assis et al., 2000).

$$\text{UPME / mL} = \frac{(\text{mL of NaOH})(\text{molarity of NaOH})(1000)}{(\text{time})(\text{mL of sample})} \quad (1)$$

## 2.4 Effect of NaCl and pH on PME activity

The effect of sodium chloride on the E-PME activity was studied in the enzymatic reaction using different concentrations of NaCl (0.2, 0.4, 0.6, 1.0 and 1.5 mol/L) in pectin substrate. Likewise, the pH was varied within the same reaction (6.5, 7.0, and 7.5) to use for the measurement of activity during the subsequent experiments.

## 2.5 Thermal inactivation of PME crude extract

The dialyzed crude enzyme extract was treated using different times (0 to 40 min) and temperatures (64 to 78 °C) in thin-wall test tubes (6 mm I.D.) placed in a water bath. The heating time was recorded for each sample until the desired temperature was reached. After treatment, the samples were immediately cooled using an ice water bath.

## 2.6 Kinetic parameters

The kinetic parameters for enzyme inactivation of mango PME were calculated using logarithmic reduction value (*D*) (Equation 2), the rate constant for inactivation (*k*) (Equation 3), the thermal resistance value constant *z* (Equation 4) described for a model of first-order inactivation well as the activation energy from the Arrhenius equation (*E<sub>a</sub>*).

$$\log a - \log b = \frac{t}{D} \quad (2)$$

$$\frac{b}{a} = e^{-kt} \quad (3)$$

$$z = \frac{2.303 R Ta^2}{Ea} \quad (4)$$

Where *a* is the initial activity units, *b* is the residual activity units at time *t*, *k* is the inactivation constant, *R* is the ideal gas constant (kJ mol<sup>-1</sup> K<sup>-1</sup>), *T<sub>a</sub>* is the average of the experimental absolute reference temperature (K) and *E<sub>a</sub>* is the activation energy.

Obtained data was analyzed using ANOVA and the mean comparison was carried out by Tukey's test at *P* < 0.05.

## 3 Results and discussion

### 3.1 Enzymatic mango extracts

The crude extract obtained from Ataulfo variety mango bagasse pulp showed an enzyme activity of 52 UPME/100 g of wet pulp under the measurement conditions (pH 7.5 and 0.2 mol/L NaCl, 30 °C). The enzymatic activity of this variety of mango was low and similar to Keitt variety mango puree where levels of activity up to 12.6 ± 1.1 UPME/g have been reported (Banjongsinsiri et al., 2004; Jamsazzadeh Kermani et al., 2015). Low activity (4.5 UPME/100 g of pulp) was found in Zebda variety mango. However, it depends on the fruit tissue analyzed, where higher levels of activity were found in the skin when compared to the pulp by 8-folds (Labib et al., 1995).

### 3.2 Thermal inactivation of PME

Thermal inactivation treatments of E-PME were carried out in a temperature range of 64 to 78 °C using a water bath. At this temperature range, the isothermal inactivation of PME showed different behaviors in the inactivation of the enzyme versus time. At temperatures below 70 °C nonlinear PME inactivation pattern was recorded and at temperatures above 70 °C, a linear inactivation pattern was observed as described by a first order kinetic model for the inactivation of the enzyme

(Figure 1). This suggests the presence of PME thermolabile and thermostable fractions. Such inactivation profile of PME has been reported also for orange, banana, and carrot extracts and is associated with the presence of isoenzymes (de Assis et al., 2000; Labib et al., 1995; Ly-Nguyen et al., 2003; Seymour et al., 1991; Vercet et al., 1999). On a technological level, the thermostable fractions are the most important factors used to determine the pasteurization values. For example, Versteeg et al. (1980) showed that 0.8 minutes at 90 °C is necessary to inactivate 99% of high molecular weight PME, which represents the thermostable fraction of navel orange PME.

In the transition between the two heat inactivation behaviors observed, at 68 °C biphasic inactivation is demonstrated. This behavior is due to the presence of two inactivation patterns, which are shown on the same plot (Figure 2). The first section of the curve is associated with the inactivation of the thermolabile fraction during the first 10 min of treatment. The second section of the curve is associated with the thermostable fraction, maintaining residual activity for at least 40 min after heat treatment (Figure 2). This biphasic behavior characteristic has also been described in orange (Seymour et al., 1991; Vercet et al., 1999) and carrot PME (Ly-Nguyen et al., 2003). In some published studies it has been implemented as a selective method for the inactivation of thermolabile PME isoenzymes (Ly-Nguyen et al., 2003; Seymour et al., 1991; Vercet et al., 1999).

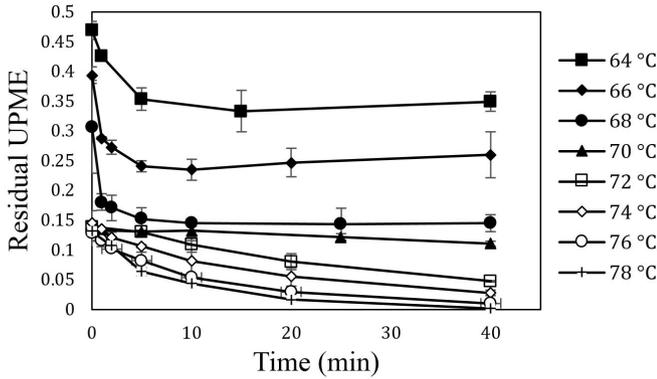
Heat treatments at temperatures above 70 °C, generally show linear patterns of E-PME inactivation (Figure 3), resulting in D values reported in Table 1, with low residual activities for the treatment times studied (17% of the total residual activity). These low residual activity values correspond to those reported in the literature for different varieties of mango (Giovane et al., 1995; Jamsazzadeh Kermani et al., 2015).

The z value calculated in the temperature range from 72 to 78 °C for PME was 9.44 °C obtained from the constants of inactivation using the Arrhenius equation (Table 1). This was a lower value than 18.5 °C reported by Labib et al. (1995) who studied a different mango variety and different kinetics approach working with temperatures below 70 °C. The activation energy for thermal inactivation calculated using the Arrhenius equation (Hernández-Botello et al., 2014) (Figure 4) was 245.6 kJ mol<sup>-1</sup> K<sup>-1</sup>, which could not be compared with other values due to the lack of information, however this value is similar to the values found in similar studies carried out with oranges (Duvetter et al., 2009).

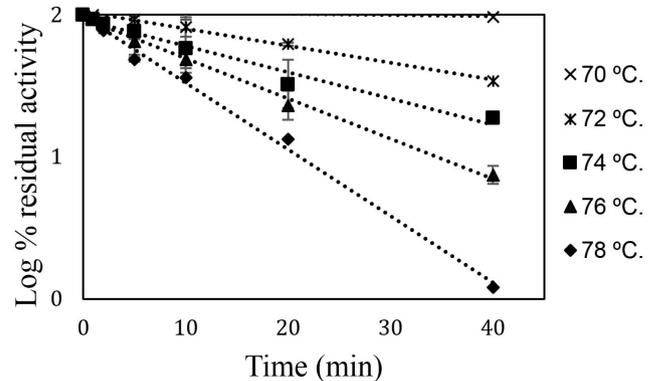
**Table 1.** Calculated D and k values of Ataulfo mango.

Temperature (°C)	D value (min)	Inactivation constant (k)
72	82.6 ± 2.22 <sup>a</sup>	0.028 ± 0.0007 <sup>a</sup>
74	55.3 ± 2.21 <sup>b</sup>	0.041 ± 0.0016 <sup>b</sup>
76	34.7 ± 5.24 <sup>c</sup>	0.067 ± 0.0103 <sup>c</sup>
78	19.0 ± 3.34 <sup>d</sup>	0.123 ± 0.0217 <sup>d</sup>

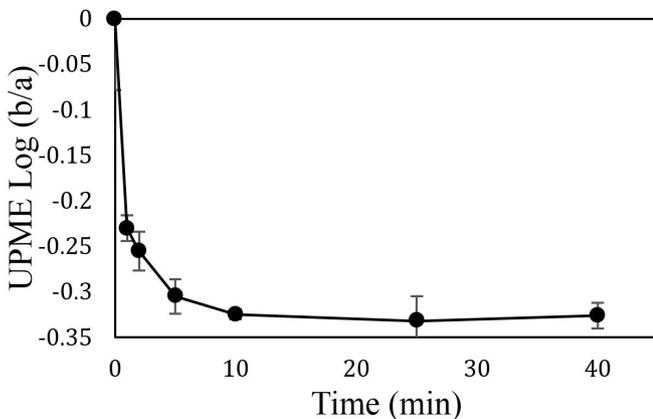
Different letters mean statistical significant difference (p<0.05).



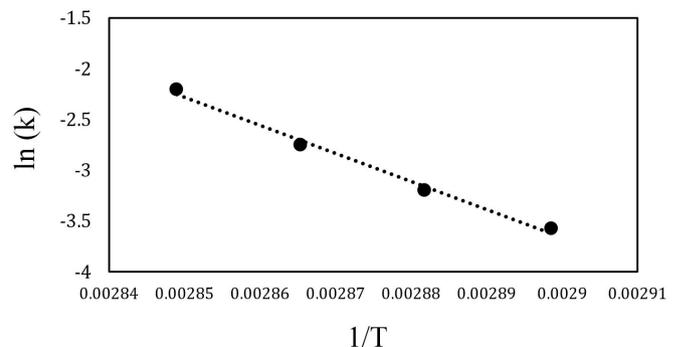
**Figure 1.** Thermal inactivation of mango E-PME.



**Figure 3.** Inactivation of mango TME-PME.



**Figure 2.** Thermal inactivation of mango E-PME at 68 °C.



**Figure 4.** Arrhenius plot for inactivation of PME.

## 4 Conclusions

In this study, two inactivation patterns of mango E-PME associated with the presence of different thermal stabilities were reported. Inactivation behavior described by a first order kinetic model for mango TME-PME fraction at temperatures above 70 °C showed a linear inactivation pattern obtained with a z-value of 9.44 °C in a temperature range of 72-78 °C. Results suggest the presence of minimal residual PME activity with a treatment time of 40 min at 78 °C with a two logarithmic cycles inactivation reaching values near to complete inactivation. Likewise, the mango TME-PME fraction represented 17% of the total mango E-PME, an important percentage to take into account for conventional or alternative inactivation treatments.

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