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Acerola's pectin methylesterase: studies of heat inactivation

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Abstract

The effect of temperature on the activity of acerola's pectin methylesterase (PME) was studied to determine its heat-inactivation. The acerola's pectin methylesterase (PME; EC: 3.1.1.11) is very stable at 50°C (10% loss of activity in 100 min) and needed 110 min for its inactivation at 98°C. These values are much higher than the ones required for inactivation of the citrus PME, that has been reported as being equal to 1 min at 90°C. Heat-inactivation of PME was shown to be nonlinear, suggesting the presence of fractions of PME with differing heat-stabilities. The times to inactive the enzyme at 98, 102 and 106°C were 110, 10 and 2.17 min, respectively. The Z value (the rise in temperature necessary to observe a ten times faster heat-inactivation) was 4.71°C.

Keywords: Pectin methylesterase; Inactivation enzyme; Acerola

1. Introduction

Acerola (*Malpighia glabra* L., *Malpighia punicifolia* L or *Malpighia emarginata* DC) grows from South Texas, through Mexico and Central America to northern South America and throughout the Caribbean. More recently it has been introduced in sub-tropical areas throughout the world (Asia, India, South America) and some of the largest plantations are in Brazil (Cooper, 1971; Facciola, 1990; Morton, 1987). Acerola fruit is rich in vitamin C as well as carotene, thiamin, riboflavin, niacin, proteins, and mineral salts, mainly iron, calcium and phosphorus. It has high antioxidant activity which may be due in part to its high vitamin C content.

The acerola (*Malpighia glabra* L.) is one of many tropical fruits that may assume a great economical importance in Brazil. Its main use is in the production of juices, concentrates, pulp and vitamin C. Fruit juices and concentrates are biphasic systems, made up of a liquid phase, termed "serum", and a solid phase which, in orange juice, is termed cloud (Castaldo, Lovoi, Quagliulo, Servillo, Balestrieri & Giovane 1991). Cloud retention in citrus juice is a major concern of citrus processing. Wicker and Termelli (1988) have attributed loss of cloud primarily to de-esterification by pectin methylesterase (PME). The action of PME is to remove the methoxyl groups from methylated pectic substances (Srivastava, Nighojkar & Kumar, 1996; Whitaker, 1984). The interaction of the resulting pectic acid complexes with calcium ions to form insoluble calcium pectate, causes loss of cloud with consequent clarification of the juice.

Thermal treatment is the most common technology that has been used to solve the problem of undesired juice clarification. In industries, the inactivation time for citrus PME has been reported as being equal to 1 min at 90°C by Eargeman and Rouse (1976) and Versteeg, Rombouts, Spaansen and Pilnik (1980). It is known that the heat treatment can affect aroma and flavour of juices and concentrates. The main purpose of this study is to determine whether thermal treatment is suitable to inactive acerola's PME and to calculate the D and Z values for heat-inactivation of PME.

2. Materials and methods

2.1. Preparation of pulp

The acerola fruits were collected from several trees. In each tree they were collected at different points of the same tree to guarantee the representability of the sample, because the seasonal time has an influence on

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fruit development. The sample was obtained by mixing those fruits collected. The fruits were divided according to the stages of their ripening, colour, diameter, weight and volume. The pulp was obtained by passing the fruits through a de-pulper with a propeller-blade.

2.2. Extraction of the PME

The enzyme was extracted, at 4°C, by using borate-acetate buffer 50 mM, pH 8.3, containing 0.60 mol/l NaCl solution. The ratio of acerola material to extractant was 1:3 (g/ml). The homogenate was squeezed through two layers of gauze and the extract was centrifuged at 10,000×*g* to remove the solid particles (Körner, Zimmerman & Berk, 1980). The precipitate was discarded. The supernatant was brought to 70% saturation by addition of solid ammonium sulfate and centrifuged at 10,000×*g* for 10 min after standing for 1 h. The precipitate was resuspended in borate-acetate buffer in the ratio of 1:3 (w/v).

2.3. Determination of PME activity

PME activity was measured titrimetrically by determining free carboxyl groups formed as a result of enzyme action on pectin. The reaction mixture was composed of 15 ml of the 0.25% citrus pectin solution, 0.15 mol/l NaCl, 0.5 ml of enzyme concentrate and final volume was adjusted to 30 ml with distilled water. The amount of 0.1 M NaOH required to maintain the reaction mixture at pH 8.3 (27±2°C) was measured by the method described by Kertesz (1955). One unit of PME was defined as the amount of enzyme which released 1 μmol of carboxyl groups/min. PME activity was calculated by the following formula (Balaban, Arreola, Marshall, Peplow, Wei & Cornell 1991):

$$\text{PME units/ml} = \frac{(\text{ml NaOH})(\text{Molarity of NaOH})(1000)}{(\text{time})(\text{ml sample})}$$

2.4. Thermal inactivation of PME

The samples of concentrated PME in test tubes (selected to be equal in weight and size) were incubated in a water or oil bath at different temperatures and for various times. After the heating process, the tubes were cooled in melting ice and the residual activity was measured. Average values of duplicates (which differed less than 5%) were calculated.

3. Results and discussion

The heat stability of PME at different temperatures is shown in Fig. 1. The PME submitted at 50 and 80°C retained 90% of its activity during 100 and 50 min,

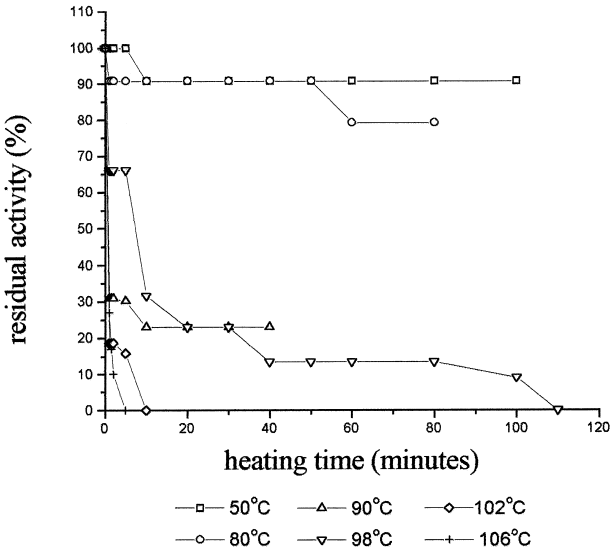

Fig. 1. Heat stability of PME from acerola at 50, 80, 90, 98, 102 and 106°C.

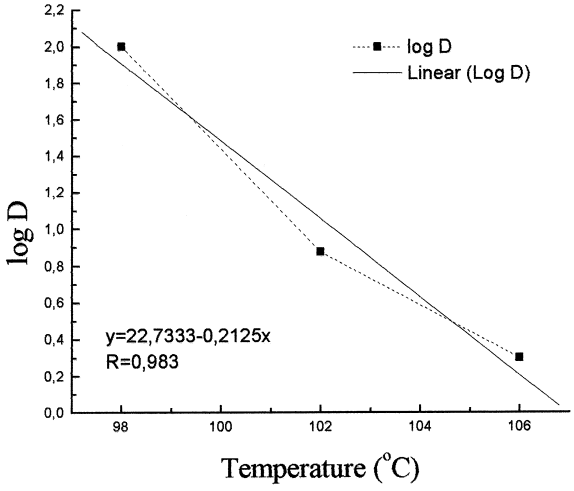

Fig. 2. Calculation of the *Z* value.

respectively. The heating times necessary to total inactivation of PME at 98, 102 and 106°C were equal to 110. 10 and 2.17 min, respectively, and the *D* values (time to inactive 90% of the enzyme), determined at the same temperatures, were equal to 100, 7.5 and 2 min, respectively. The *Z* value (the rise in temperature necessary to observe a ten times faster heat-inactivation) was 4.71°C (Fig. 2). One of the most thermally tolerant methylesterases from citrus juice has been reported by Cameron and Grohmann (1996), and this enzyme retained 49.2% relative activity after 60 s incubation in a 95°C water bath.

The *D* values obtained are very high when compared to the *D* values at 90°C from citrus PME in orange juice pulp (Wicker & Temeli, 1988), estimated to be 0.225 and 32 s, respectively, for the heat-sensitive and heat-stable fractions.

Our results show that the heat-inactivation was non-linear (Fig. 2). One of the possible explanations is that these samples have enzyme systems with different thermal stabilities, possibly due to enzyme aggregates formed during heat-inactivation or isoenzymes (heat-stable and heat-labile isoenzymes; Nath, 1995).

The results also show that heating times required to inactive the PME acerola are much higher than the time required by other citrus PME. Therefore, for acerola juice industrialization, alternative technological approaches will probably be necessary to solve the problem of juice clarification.

In summary, the results show that acerola has a PME enzyme too stable for utilization in an industrial juice process. The inactivation of acerola's PME was non-linear, possibly suggesting the presence of fractions of PME with different heat-stabilities and it showed higher thermal inactivation tolerance than other sources of PME, such as orange juice.

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