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Thermostability of three pectinesterase isoenzymes in tomato fruit

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The thermal stability of three forms of pectin methylesterase purified from tomato was evaluated at pH 4·5. The time-dependent heat-inactivation curves for the three forms exhibit exponential behaviour in the temperature range between 70°C and 90°C. The determination of D_T and Z parameters displays a different heat-inactivation kinetic among the three isoenzymes. In particular, two forms (PME_1 and PME_3) show similar behaviour with a Z value of 24°C, whereas the third form (PME_2) shows a Z value of 15°C.

INTRODUCTION

The stabilisation of fruit and vegetable juices is an essential technological step in order to obtain products of good texture and reproducible physico-chemical properties (Rothshild *et al.*, 1975). The main goal of this technology is the development of a process which prevents the growth of microorganisms and eliminates the endogenous enzymatic activity, leaving unmodified the nutritional and texture properties of the juice. To achieve these results, the control of pectolytic enzymes is of prime importance. In particular, the pectin methylesterase (PME) activity is shown to be responsible for cloud destabilisation of fruit juices (Baker, 1977). PME (EC 3.1.1.11) hydrolyses the methylesters of polygalacturonic acid and is an ubiquitous plant enzyme (Nagel & Patterson, 1967) which is bound to the cell wall and is involved in the fruit–ripening process (Zauberman & Schiffmann-Nadel, 1972; Solomos & Laties, 1973; Grierson *et al.*, 1981). The biochemical properties of this enzyme have been investigated in material from various sources (Versteeg *et al.*, 1978; Giovane *et al.*, 1990; Fayyaz *et al.*, 1993; Mcdonald *et al.*, 1993) and the primary structure of the tomato enzyme has been determined (Markovic & Jornvall, 1986).

From a technological point of view, the thermal inactivation of PME is an important subject. The heat treatment of juices is a process commonly used for their stabilisation. In fact, this technique (Whitaker, 1972) has a double purpose, i.e. the sterilisation of the product and the inactivation of PME, since the temperature of enzyme inactivation is consistently higher than that of pasteurisation. The thermal resistance of PME was investigated in orange, where different behaviour was found among its isoenzymes (Versteeg *et al.*, 1980). Similar studies were also performed in tomato, although not with regard to the difference among isoenzymes.

Because at least three different forms of PME have been found in tomato (Giovane *et al.*, 1994), it is considered that a study of the thermal inactivation of these forms could be helpful in optimising a process aiming to stabilise the tomato products.

MATERIALS AND METHODS

The purification procedure of the three forms of pectin methylesterase from tomato is described elsewhere (Giovane *et al.*, 1994).

Evaluation of D_T and Z parameters

The thermostability of the three PME forms was determined following the enzymatic activity as a function of temperature at various times of treatment. The kinetic parameters D_T and Z were calculated according to Stumbo (1973).

The decimal reduction time was determined using the following equation:

$$D_T = t/(\log a_i - \log a_f)$$

where a_i is the initial activity, a_f is the remaining activity after the treatment and t is the time (in seconds) of thermal treatment at a given temperature. The D_T parameter indicates the time necessary to obtain a decimal

reduction of the enzyme activity. The Z parameter is obtained by plotting the D_T values on a log scale against the corresponding temperatures, and the thermal resistance curves were established by applying least-squares analysis. The thermal inactivation experiments were carried out in the range 70–90°C for 5–60 s.

Thermal inactivation and PME activity determination

Samples (30 μl) of pure PME isoform in 20 mM Tris–HCl, pH 7·5, 50 mM NaCl and 5 mM mercaptoethanol were poured into the bottom of a 400-μl plastic tube by a microsyringe and the tube was immediately capped. The tube was pre-heated at a fixed temperature for 5 min and the time was measured starting from the sample addition. After each time interval, the tube was chilled in an ice bath and the enzymatic activity assayed. Each determination was done in triplicate.

PME activity was determined titrimetrically (Vas *et al.*, 1967), using an automatic pH-stat (Crison model TT2050), in a thermostated cell. Pectin (70% methylated, Sigma) was used as substrate in 1% solution containing 0·15 M NaCl, pH 4·5 and 20 μl of PME, in a final volume of 25 ml at 30°C. The titrating solution (NaOH 0·05 M) was kept under a nitrogen stream to avoid carbonation. The PME activity was expressed in units (μmoles of H^+ produced per min in 1 ml of incubation mixture) according the following equation:

$$u = \frac{(Vs - Vb) \times M_{NaOH} \times 1000}{V \times t}$$

where:

Vs = NaOH used to titrate the sample (ml),
Vb = NaOH used to titrate the blank (ml),
M_{NaOH} = NaOH concentration (molarity),
V = incubation mixture (ml), and
t = time of analysis (min).

RESULTS AND DISCUSSION

Three forms of PME, named PME_1, PME_2 and PME_3, were obtained from tomato fruit (var. S. Marzano) according to (Giovane *et al.*, 1994). The PME forms were subjected to thermal denaturation in order to investigate their resistance to temperature. As shown in Fig. 1, the thermal inactivation of PME_1 was tested at four different temperatures in the range 75–90°C. This form appears to be almost completely inactivated at 80°C for 60 s. The behaviour of PME_2 was investigated in a temperature range 75–85°C (Fig. 2) because of its lower heat resistance at temperatures higher than 85°C. Finally PME_3 activity was tested in the range 70–85°C (Fig. 3). All three forms revealed a heat-inactivation kinetic which displays an exponential behaviour. PME_1 and PME_3 showed a similar heat-denaturation pattern which differs from that displayed by PME_2.

From the data reported in Figs 1, 2 and 3, the thermal reduction values were determined according to the eqn (1) and the heat-resistance curves for each PME are reported in Fig. 4. The data clearly show different inactivation kinetics of the three PME forms with respect to the thermal treatment. In particular, PME_2 is quite different from PME_1 and PME_3, although PME_1, having a higher value of thermal reduction time, seems to be more resistant than PME_3. On the other hand, PME_2 shows a lower heat-resistance than the other PME forms at temperatures higher than 87°C, while it seems to be more resistant at temperatures lower than 85°C. The Z values (i.e. the temperature increment required for the D_T diminution of one log unit) calculated from the values reported in Fig. 4 are 23, 15 and 24°C for PME_1, PME_2 and PME_3, respectively.

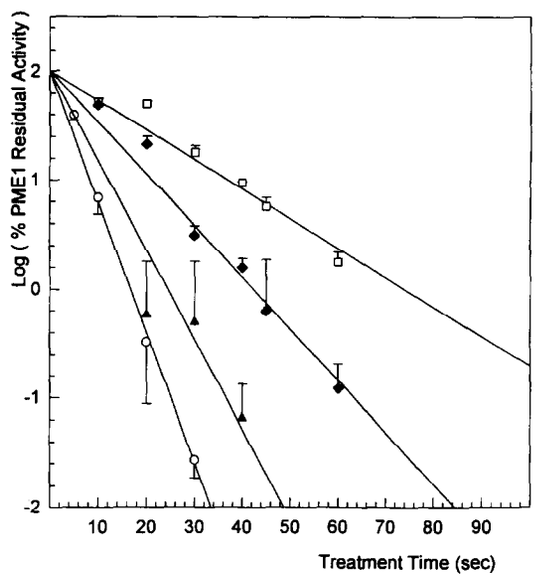

Fig. 1. Time-dependent heat-inactivation of pectin methylesterase isoenzyme 1 (PME_1). The time-dependent enzyme-inactivation was tested at four temperatures: 75°C (□), 80°C (♦), 85°C (▲), 90°C (○). Each point is the mean of five determinations.

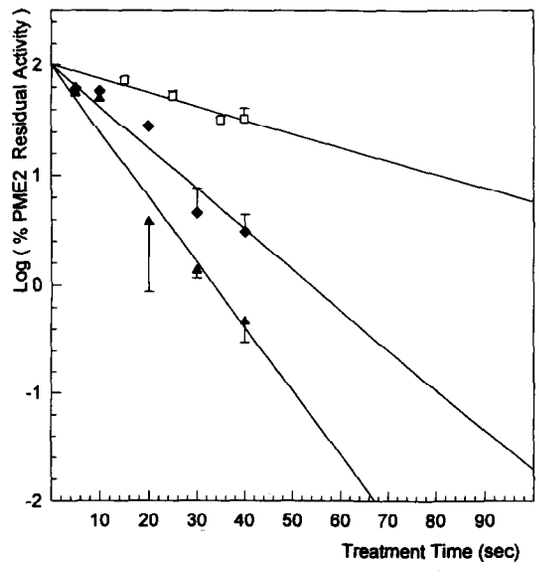

Fig. 2. Time-dependent heat-inactivation of pectin methylesterase isoenzyme 2 (PME_2). The time-dependent enzyme-inactivation was tested at three temperatures: 75°C (□), 80°C (♦), 85°C (▲). Each point is the mean of five determinations.

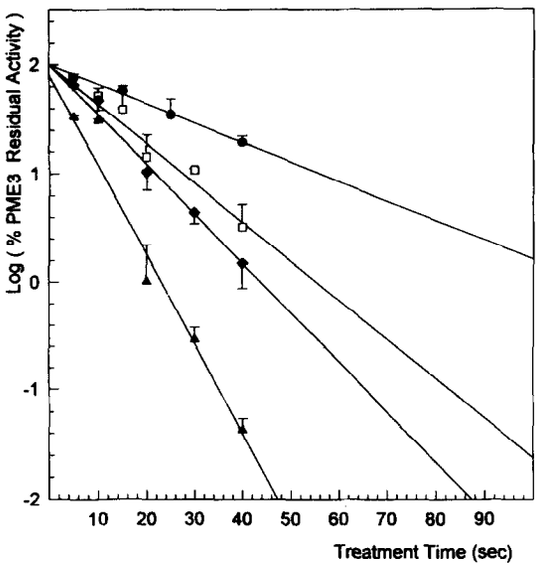

Fig. 3. Time-dependent heat-inactivation of pectin methyl esterase isoenzyme 3 (PME_3). The time-dependent enzyme-inactivation was tested at four temperatures: 70°C (●), 75°C (□), 80°C (♦), 85°C (▲). Each point is the mean of five determinations.

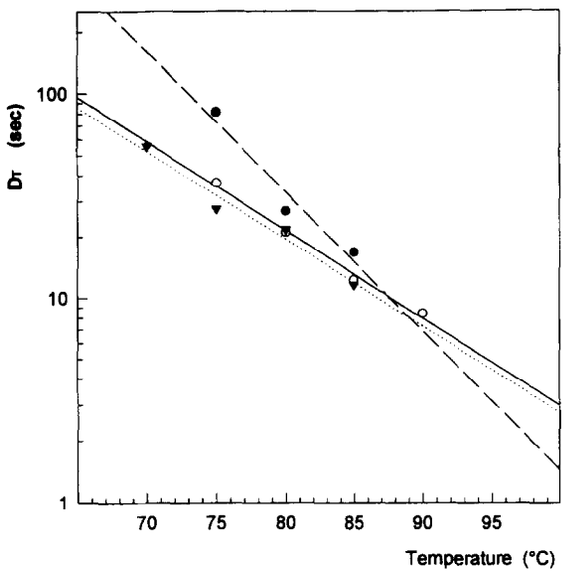

Fig. 4. Dependence of the decimal reduction time (D_T) of the three forms of pectin methylesterase as function of temperature. PME_1 (○), Z = 24°C; (●), Z = 15°C; PME_3 (▼), Z = 24°C.

These values are consistently higher than those reported by Nath *et al.* (1983), who found a value of 9°C at pH 4·5, whereas they are very similar to those found by De Sio *et al.* (1995). These last authors found a biphasic behaviour when plotting D_T values versus temperature, with a Z value of 13°C for temperatures lower than 78°C and of 29°C for temperatures higher than 78°C.

The differences between these results could be explained by considering that the results here reported are obtained from purified isoforms of PME while the results of Nath and De Sio *et al.* are from the whole tomato juice. Furthermore, due to the small sample volume employed in our assay, the time employed for warming up and cooling down steps is practically insignificant. If the presence of PME isoforms is a general rule in fruits and vegetables (Castaldo *et al.*, 1989; Rillo *et al.*, 1992), the measurement conditions utilising the TIT tube method (Nath) could inactivate the less resistant (at low temperature) PME forms mainly in the warm-up step. In fact, because of the large section of the sample, as in the case of Nath's experiments, the heat penetration takes time. Hence the Z value found is essentially due to the two PME forms which are more resistant at higher temperatures. The data reported by De Sio *et al.* show a biphasic behaviour of PME thermal inactivation and, considering our result, could be ascribed to a sum of two phenomena. In fact, at lower temperatures (<78°C) the more resistant form is PME_2, hence the total PME activity is chiefly due to this isoenzyme. On the other hand, at higher temperature, the Z value is determined by PME_1 and PME_3 that are more resistant at these higher temperatures. In this case the warm-up effect is minimized because of the smaller sample section.

The presence of multiple forms of PME in fruits and vegetables could be a technological problem if these forms have different heat-resistance as happens in the tomato. In fact, the PME forms characterised by higher Z values are those more quickly inactivated at lower temperatures, whereas they display higher resistance at higher temperatures. This behaviour is important in treatments such as High Temperature Short Time technology (HTST), inasmuch as the presence of PME isoenzymes with high heat-resistance could counteract the benefit of thermal treatment. Moreover, as reported elsewhere (Giovane *et al.*, 1994), the presence of heat-resistant PME forms in tomato (PME_1 and PME_3) represents about 70% of total PME activity therefore their complete inactivation is essential for effective cloud stabilisation.

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