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Thermal resistance of tomato polygalacturonase and pectinmethylesterase at physiological pH

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Abstract The kinetic parameters of heat-induced inactivation of tomato fruit pectic enzymes in a partially purified form have been calculated. Polygalacturonase I (PGI), polygalacturonase II (PGII) and pectinmethylesterase (PME) were separated by means of gel permeation chromatography and their thermoresistance in 50 mM citrate buffer, pH 4.0, 0.4 M NaCl calculated. All the enzymes showed first-order kinetics of inactivation. PGII *D* values ranged from 2.14 min at 64 °C to 0.24 min at 73 °C. Its *z* value was calculated to be 9.4 °C. PGI *D* values ranged from 15.9 min at 86 °C to 0.46 min at 95.4 °C. Its *z* value was calculated to be 5.6 °C. PME *D* values ranged from 7.6 min at 66.4 °C to 0.20 min at 74.5 °C. Its *z* value was calculated to be 5 °C.

Key words Pectic enzymes · Thermal resistance · Tomato fruit

Introduction

Viscosity is one of the most important quality characteristics of tomato juice [1]. High viscosities are preferred by consumers. Tomato juice viscosity is greatly dependent upon chemical composition, physical arrangement and the total amount of pectic substances [2, 3]. Degradation of pectic substances by endogenous pectolytic enzymes lowers juice viscosity and diminishes the quality of the product [4, 5]. There are two main pectolytic enzymes responsible for the degradation of pectic substances in tomato juice: pectinmethylesterase, PME (E.C. 3.1.1.11) and endopolygalacturonase, PG (1.2.1.15). PME catalyses the hydrolysis of the methyl ester groups of pectin, resulting in the de-esterification of pectin. PG catalyses the hydrolytic cleavage of α-1,4-hydrolytic bonds of polygalacturonic acid chains [6]. PME enhances the action of PG on pectic substrates because this enzyme has greater catalytic activities against demethylated substrates [7].

There are, at present, two types of treatments used for tomato juice preparation: the so-called cold break and hot break treatments [8]. In the "cold break" procedure, temperatures of around 60 °C are used to avoid colour changes and ascorbic acid losses. At these temperatures the pectin-degrading enzymes are active, leading to large amounts of cellulose fibrils and cell wall being liberated into the serum phase. These fibrils increase the consistency of the final product because they become entangled. The "hot break" methods relies on early inactivation of PME and PG to obtain high-viscosity products. This is usually achieved by quick treatment at temperatures in the range of 82–104 °C [8].

The use of high temperatures to inactivate enzymes can have a negative effect on other important characteristics of tomato juice, such as colour, flavour and nutritional value [9]. It is therefore important to adjust the heat treatment conditions (time and temperature) to inactivate most of the PG and PME with the minimum possible damage to the desirable characteristics of the tomato juice. This requires a good knowledge of the thermal resistance parameters (*D* and *z* values) of both PG and PME. Although there are a number of papers dealing with the heat resistance of tomato PG [5, 10–16] and PME [5, 17–23], *D* and *z* values for PG are lacking and those published for PME are either not very reliable due to methodological weaknesses [23], or have been measured at pH values very different from that of tomato juice [22], or show strong divergences [20, 22]. The aim of the present work was to calculate *D* and *z* values for tomato PG and PME at pH 4.0, the

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physiological pH of tomato, to allow good estimation of the PG and PME inactivation which might be expected from any heat treatment.

Materials and methods

Materials. Ripe tomato fruits (Daniela F1) were purchased at a local market. PG and Pectin from Citrus fruits were obtained from Sigma.

Enzyme inactivation parameters. Thermal resistance of the enzymes are expressed in terms of D and z values. D value is defined as the time of heating at constant temperature (subscript; in °C) required to inactivate 90% of the original enzyme activity. z value is the increase in temperature required for the D value to be 10-fold lower.

Enzyme extraction. PG and PME were extracted from the pericarp tissue of ripe tomato fruits. Tissue (2 kg) was cut into small pieces and washed twice with 2 l of cold water. Water was eliminated by passing the tomato pieces through a 1.2-mm sieve. Then 2 l of cold 10 mM citrate buffer, pH 5.5, was added to the pericarp pieces and homogenized with a household blender. The homogenate was centrifuged at 10,000 g for 40 min. The pellet (450 g) was recovered, redissolved in 500 ml of 10 mM citrate buffer, pH 5.5, and centrifuged again at 10,000 g for 40 min. The pellet (300 g) was redissolved in 2 l of cold 50 mM citrate buffer, pH 5.5, plus 1.7 M NaCl and vigorously agitated in a reciprocal shaker for 90 min at 4 °C. This solution was centrifuged at 10,000 g for 40 min. The supernatant (1.85 l) was concentrated to 125 ml in a hollow fibre concentrator (Model DC2, Diaflo fiber PM3; Amicon, The Netherlands), divided into aliquots, frozen in liquid nitrogen and stored at − 30 °C.

Separation of PME and PG isoenzymes. A 15-ml aliquot of the frozen concentrated crude extract was thawed and further concentrated to 5 ml by ultrafiltration with Microsep tubes, 10 K "cut-off" (Northborough, USA). The further concentrated extract was applied to a Sephacryl S-200 column (2.6 × 96 cm) equilibrated with 50 mM citrate buffer, pH 5.5, 1.7 M NaCl. The column was eluted at a flow rate of 11 ml/h. Fractions of 10 ml were collected.

PG assay. PG was assayed by measuring the increase in reducing groups of a polygalacturonic acid substrate using the method described by Lever [24]. The reaction was performed at 40 °C for 1–6 h and was started by adding the PG preparation to 0.25 ml of a 0.4% water solution of polygalacturonic acid. In the thermal resistance studies, 0.25 ml of the enzyme solution (50 mM citrate buffer, pH 4.0, 0.4 M NaCl) was used and, for column monitoring, 1 µl of eluate dissolved in 0.25 ml of the above-cited buffer. PG activity is expressed in units, defined as µmoles galacturonic acid produced per minute per millilitre.

PME assay. PME was assayed by acid–base titration. The assay test consisted of 20 ml of a 0.5% solution of pectin in 0.15 M NaCl solution adjusted to pH 7.0. The reaction took place at room temperature and was initiated by adding the HPME solution (20–200 µl). PME activity is expressed as mEq NaOH $min^{-1}ml^{-1}$.

Heat treatments. Heat treatments were performed using a TR-SC thermoresistometer [25]. Tomato extracts were injected into the treatment chamber containing 23 ml of the treatment medium at the present temperature. Treatment medium consisted of 50 mM citrate buffer, pH 4.0, 0.4 M NaCl. Periodically, 400-µl aliquots were removed, immediately cooled on ice-water and assayed for PME or PG activity.

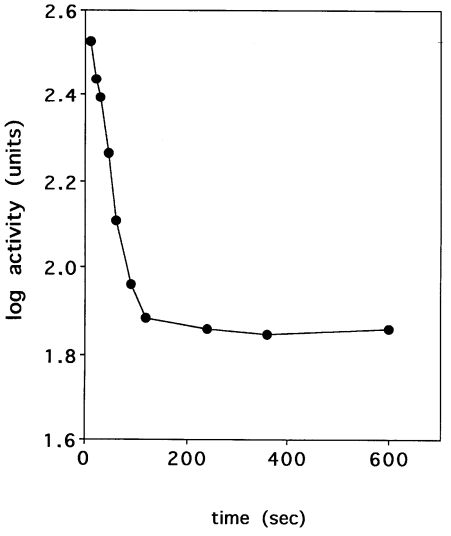

Fig. 1 Heat-induced inactivation of polygalacturonase (PG) from a crude tomato extract at 72.5 °C. PG activity (●)

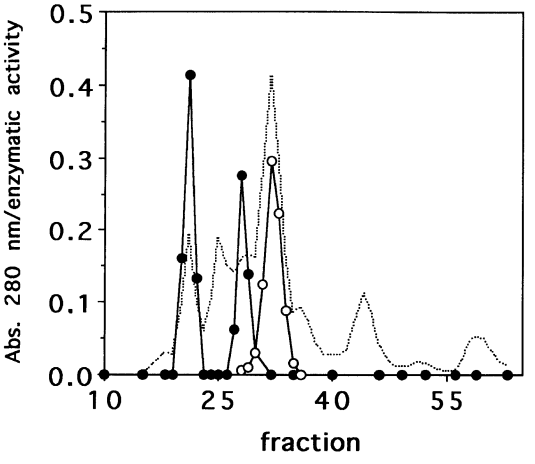

Fig. 2 Gel permeation chromatography, on Sephacryl S-200 of a crude tomato extract. *Dotted line* shows absorbance at 280 nm. Polygalacturonase (*PG*) activity/28,000 (●) ; Pectinmethylesterase (*PME*) activity/1000 (○)

Results

Plots of the log of residual activity of the crude extracts versus time of heating at 72.5 °C (Fig. 1) confirmed the presence of two isoforms of PG with different thermostability, as previously observed by a number of authors [11–16]. From these plots, D value calculations for both isozymes can be made [26], but these estimates are not very reliable, particularly that for the most thermosensitive isozyme.

Gel permeation of the crude extracts (Fig. 2) allowed the resolution of PG activity in two peaks (PGI and PGII). PME eluted as a single peak which overlapped

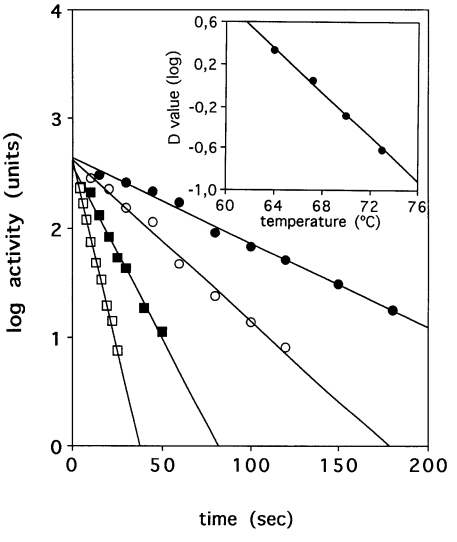

Fig. 3 Heat treatment of Polygalacturonase II (*PGII*) at 64 °C (●); 67.2 °C (○); 70 °C (■) and 73 °C (□). *Inset*: plot of log D_t, as obtained from these time courses of enzyme inactivation, versus temperature (T), to obtain the z value

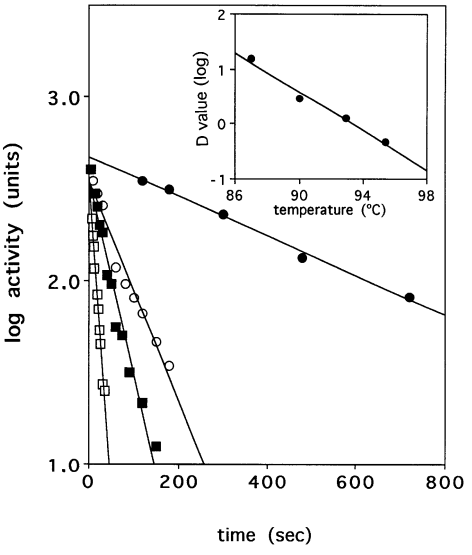

Fig. 4 Heat treatment of Polygalacturonase I (*PGI*) at 86 °C (●); 90 °C (○); 92.9 °C (■) and 95.4 °C (□). *Inset*: plot of log D_t, as obtained from these time courses of enzyme inactivation, versus T, to obtain the z value

slightly with the low-molecular-weight PG isoform, PGII.

Heating of PGII (Fig. 3) and PGI (Fig. 4) showed first-order inactivation kinetics. *D*-values can be calculated easily from these plots. For PGII, they range from 2.14 min at 64 °C to 0.24 min at 73 °C, from which a z value of 9.4 °C can be estimated (see inset in Fig. 3). As is well known [11–16], PGI is more thermoresistant compared with PGII, and *D* values calculated from plots in Fig. 4 (ranging from 15.9 min at 87 °C to

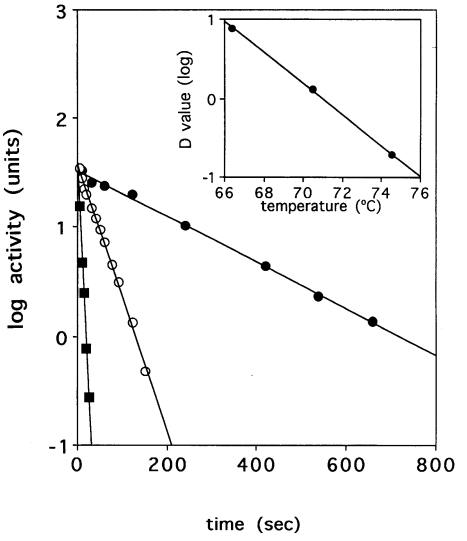

Fig. 5 Heat treatment of PME at 66.4 °C (●); 70.5 °C (○) and 74.5 °C (■). *Inset*: plot of log D_t, as obtained from these time courses of enzyme inactivation, versus (T), to obtain the z value

0.46 min at 95.4 °C) allow an estimation (inset of Fig. 4) of the z value of 5.6 °C.

The gel permeation fraction with the highest PME specific activity showing no detectable PG activity was heat treated at 66.4, 70.5, and 74.5 °C. Plots of the log of residual activity versus time also reveal (Fig. 5) first-order kinetics of inactivation. *D* values range from 7.6 min at 66.4 °C, to 0.2 min at 74.5 °C, allowing the calculation (see inset of Fig. 5) of the z value of 5 °C.

Estimations of PGI and PME z values from plots of residual activity after heating crude extracts in the temperature intervals of 66.4–74.5 °C and 86–97.3 °C (data not shown) yielded the same values as obtained for these enzymes in the partially purified preparations.

Discussion

Our data show clearly that the cold break procedure does not destroy PME and PGI activities and destroys that of PGII only slightly.

The presence of various PME isozymes [22, 23, 27, 28] with different degrees of glycosylation [29] and thermostability [22, 23] in tomato fruits has been reported. Their chromatographic separation requires the use of affinity chromatography since they do not show significant differences in molecular weight [29]. It is not surprising, therefore, that all the activity eluted in a single peak in our gel permeation experiments. It is surprising, nevertheless, not to have found biphasic courses of PME inactivation in the crude extracts. This may be due either to the presence of a single isozyme in the fully ripe state of the Daniela F1 hybrid tomatoes used here, or to the different heat treatment conditions

used, particularly with regard to the pH and the buffer composition.

The heat resistance of PME, as published by the group of Castaldo [22, 23], substantially differs from data here reported. D_{81}, which they report for tomato juice (0.17 min), is an order of magnitude greater than the value which can be estimated from our data. D_{80} at pH 4.5, published by Nath et al. [20] for tomato of the cultivar Pusa-Ruby, is even higher. In addition, z value estimations by the Castaldo group are (both for the purified isoenzymes and whole tomato paste) threefold to sevenfold higher than that measured in this study, which is more in agreement with the value of 9 °C reported by Nath et al. [20]. There may be differences in the degree of glycosylation of the PME of this study and that of Castaldo's group, because of the different cultivars used and their degree of ripening; these factors may contribute to the discrepancies in the results obtained. In addition, the hindrance to heat exchange introduced in the experiment of Castaldo's group by the use of plastic bags, or plastic tubes for the heat treatments must substantially contribute to these divergences.

The existence of two forms of PG of very different stability in tomato has been known since 1973 [11]. The relationship between both forms of the enzyme and their physiological role have been an active subject of research for the last 10 years. PGII is composed of a single polypeptide chain [13, 30, 31]. Isoforms of PGII with different degrees of glycosylation have been described [13, 30, 32] but differences in their thermostability have not been reported. We do not know if our preparations contain more than one PGII isozyme, although if they do they must have very similar thermoresistance since semilog plots of residual activity versus heating time are linear (see Fig. 3).

PGI is a dimer composed of a catalytic subunit identical to PGII and a second subunit [31–33], called β, the function of which is still unknown.

The β subunit does not appreciably change the catalytic properties of PG but dramatically enhances its thermal resistance and alters its extractability from tomato homogenates [34]. Some authors claim that the β subunit anchors the catalytic subunit to its substrate in the cell wall [15], and that it plays a role in regulating the extent of pectin solubilization and depolymerization in fruit ripening [35, 36], whereas others think that PGI is an artefact of the extraction procedure [37–39]. This question is of little importance to the food technologist although, if PGI is an artefact, the search for technological processes that avoid the interaction between PGII and the β subunit could be of great interest. As already mentioned, available heat resistance data have revealed that PGI is much more resistant to heat inactivation compared to PGII but without measurement of the kinetic parameters of the inactivation reactions comparisons of resistance at different temperatures cannot be made easily. Our data show that at the lowest hot break temperature used industrially (82 °C), about 3.5 s is enough to reduce PGII activity to 1% of its original value (the estimated D_{82} for PGII is ≈ 1.7 s) whereas to achieve the same effect on PGI the treatment should be prolonged for more than 200 min. The low z value of PGI allows the achievement of the same degree of inactivation at 104 °C in about 1.5 s (the estimated D_{104} for PGI is ≈ 0.7 s). Since the temperature dependence of most chemical reactions is much lower, it is likely that such a treatment would produce little damage to most of the desirable physical and chemical tomato juice characteristics.

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