ELSEVIER

Food Chemistry 52 (1995) 135–138

0308-8146/95/$9.50

Thermal resistance of pectin methylesterase in tomato juice

Francesco De Sio,[a] Giuseppe Dipollina,[b] Gerardo Villari,[a] Roberto Loiudice,[a] Bruna Laratta[a] & Domenico Castaldo[a]*

[a]*Department of Tomato Products,* [b]*Department of Packaging and Food Sterilization, Stazione Sperimentale Industria Conserve Alimentari in Parma, Sede di Angri (SA), Via Nazionale 121/123 Angri (SA), Italy*

(Received 8 September 1993; revised version received and accepted 2 March 1994)

The behaviour of pectin methylesterase (PME, EC.3.1.1.11) was analysed in five new tomato cultivars for juices and tomato products. The thermal inactivation of this enzyme was evaluated in the range 73–88°C and was found to be exponential. For each cultivar examined the logarithmic values of decimal reduction times plotted against temperature had a classic biphasic pattern featuring a sudden change in slope at temperatures exceeding 78°C. The z values determined in the range 78–88°C were much higher than those calculated from the log D_T versus T°C in the range 73–88°C.

INTRODUCTION

The tomato has a particularly complex enzymatic system which is still widely studied.The enzyme pectin methylesterase (PME, EC 3.1.1.11) plays a fundamental role, not only from a physiological standpoint (Koch & Nevins, 1989; Nari *et al.*, 1991); it demethoxylates pectins and is believed to be involved in degradation of pectic cell wall components by polygalacturonase in ripening tomato fruit. Because of its discrete thermoresistance, it may cause cloud (turbidity) instability when partially inactivated in products such as pastes and/or sauces.

The literature reports (Joslyn & Pilnik, 1961; Krop & Pilnik, 1974) that these clouds undergo a process similar to fruit juices such as orange juice. In tomato products, cloud very often occurs in the top of a container or appears as a high transmitting serum layer at the centre of the container. Rarely does it occur as a very clear serum at the bottom of a container.

To expand our knowledge on cloud loss and thermal data of PME from tomatoes, it is logical to evaluate the thermoresistance of PME in some new varieties of tomatoes used to produce sauce and/or mash to determine thermal processes of stabilization (determination of parameters F, D and z).

MATERIALS AND METHODS

Plant materials and sample preparations

Five cultivars of tomato (Olter, Rancho, Spazio 1999, Zenith, Monterosso) used to produce tomato sauce and paste were selected for this study. Trials were carried out during the 1992 production season on samples provided by STAR SPA in Sarno (SA), Italy. Each sample (about 5 kg) was carefully washed, selected, chopped and pureed using strainers with 0·4 mm diameter holes.

Samples were finally homogenized in a cold Turrax incinerator for about 3 min. Aliquots (1·5 ml) brought to pH 4·2 by addition of 0·1 M HCl were placed into 60 mm × 70 mm polythene bags.These were then sealed by heat and the thermal treatments were carried out in the same bags by immersing them in an oil bath at 73, 78, 81, 83, 85 and 88°C for a time period of 5–85 s. Afterwards, the samples were immediately cooled and stored in liquid nitrogen.

The enzymic activity was evaluated after thawing the samples at 30 ± 0·2°C. The analyses were repeated on at least five samples treated singularly at the chosen treatment temperature.

Pectin methylesterase determination

Pectin methylesterase activity was determined using a 25 ml citrus pectin solution substrate (Sigma Chemical Co., DE 62·0%) at 1% (w/w) in 0·15 M NaCl at pH 4·2. Assays were set up to use from 20 μl to 5 ml of tomato juice at pH 4·2 as a source of PME activity. The enzyme's hydrolysing effect was determined by 0·1 N NaOH titre. The pectin methylesterase activity expressed in U PME of product (micromoles of acid produced per minute per ml of product) was determined by the amount of NaOH in ml needed to maintain the solution pH at the pre-established value (pH 4·2) during the entire trial (a minimum of 30 min to a

*To whom correspondence should be addressed.

maximum of 3 h). Activity measurements came from a Crison mod TT 2050 automatic titre with a 30 ± 0·2°C thermostat.

Evaluation of *F*, *D* and *z* values of PME for thermal process calculations

As in thermobacteriology, enzymatic inactivation features kinetic parameters that make it possible to compare the degree of inactivation and the thermal treatment defined by the temperature–time curve. The most important kinetic parameter is the decimal reduction time (D_T) and its dependence on temperature expressed by z. The decimal reduction time has been calculated in agreement with Stumbo (1973) by the equation:

$$D_T = t/(\log a_i - \log a_f) \quad (1)$$

where a_i is the starting activity; a_f is the residual activity that survives the thermal treatment; and t is the thermal treatment time at temperature T in minutes.

The z parameter was derived from log D_T values at different treatment times versus temperature. The z parameter indicates how many degrees the temperature must change for the decimal reduction time to be 10-fold higher or lower. Using the general thermal stabilization process from eqn (1) we have:

$$t = TIT = D_T (\log a_i - \log a_f) = D_T\, n \quad (2)$$

where TIT is the thermal inactivation time; and n is the number of decimal reductions needed to inactivate the pectin methylesterase enzyme. In this study, as reported elsewhere on citrus fruit juices (Rothschild *et al.*, 1975; Holland *et al.*, 1976), a juice was considered commercially stable when it had a residue activity equal to or less than 10^{-4} U PME/ml or $a_f = 10^{-4}$ U PME/ml of product.

The TIT is indicated by F (inactivating effect) when dealing with an ideal thermal treatment conducted at the T_r reference temperature. The concept of a thermal treatment inactivating effect, F, is useful because it measures the destructive ability of a thermal treatment and permits comparison of different treatments Two different thermal treatments featuring the same F value produce identical effects on the enzyme. F has the dimensions of time and, for each enzyme, the inactivating effect produced by contact for 1 min at the T_r temperature is chosen as the unit of measurement. For example, any thermal treatment with an F equal to 0·5 min produces the same inactivation (that is, the same number of decimal reductions) as a 0·5 min treatment at the reference temperature. Determining the reference temperature is therefore fundamental to calculate F. Since the total enzyme inactivation requires 1 min at the reference temperature (TIT = 1 min), eqn (2) becomes:

$$F = TIT_{Tr} = n\, D_{Tr} = 1 \quad (3)$$

From this relationship the D_T value at the reference temperature ($D_{Tr} = 1/n$) was calculated and the reference temperature T_r was calculated from the experimental dependence of D_T on temperature.

RESULTS AND DISCUSSION

Table 1 reports the values and range of variation seen in at least five trials, of pectin methylesterase activity found in the five cultivars examined. The findings reveal a remarkable difference in terms of PME activity occurring in the single varieties. The highest PME activity was found in the 'Monterosso' and 'Zenith' strains with a mean value nearing 43 U PME/ml of product. The lowest PME activity came from 'Rancho' with a mean value of about 15 U PME/ml. The strains 'Spazio 1999' and 'Olter' finally had a PME activity nearing 30 U PME/ml of product at a pH of 4·2. Figure 1 reports the finding of thermoresistance trials carried out in the range 73–88°C on the Rancho cultivar. Thermal inactivation of pectin methylesterase was exponential, with a major decrease in thermal resistance upon increasing the thermal treatment temperature. Similar findings were obtained for all varieties examined.

The decimal reduction time values, taken from eqn (1), presented (as depicted in Fig. 2) a classic biphasic pattern, characterized by a sharp change in the slope, and therefore z value (°C required for the slope of the curve to traverse on log cycle), at temperatures exceeding 78°C. This behaviour is extremely important in that it may cause an incorrect determination of the z parameter and therefore causes an error in the thermal treatment calculation needed to inactivate the pectin methylesterase in this product.

In fact, as seen in Fig. 2, the z value is equal to 11·2°C when determined within 73–78°C, z = 15·6°C

Table 1. PME activity in different tomato varieties at 30°C

Tomato cultivars	PME activity (U/ml)			
	Min. value	Mean value	Max. value	Standard deviation
Monterosso	42·7	43·1	44·0	0·50
Olter	29·7	30·9	32·5	1·15
Rancho	13·0	14·5	16·0	1·29
Spazio	27·4	29·3	31·8	1·57
Zenith	43·0	43·6	44·0	0·44

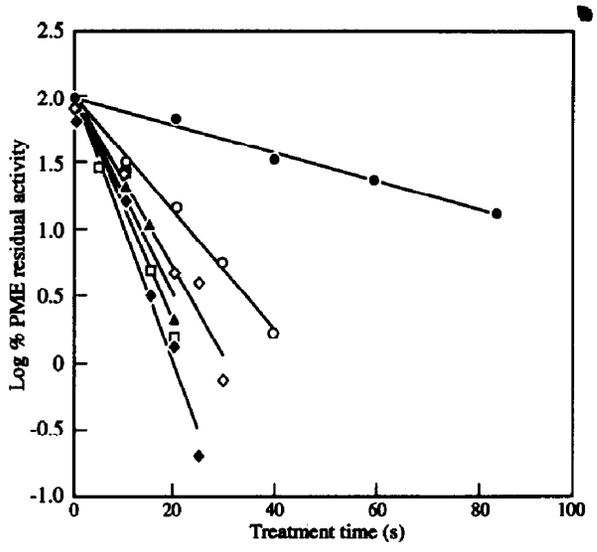

Fig. 1. Heat stability of PME in tomato 'Rancho' variety at 73°C (●), 78°C (○), 81°C (◇), 83°C (▲), 85°C (□) and 88°C (◆).

when determined within 73–88°C and finally equal to 27·8°C when determined within 78 and 88°C.

This behaviour, found in all the tomato cultivars examined, may be due to some particularly resistant isoenzyme of pectin methylesterase. Moreover, the occurrence of multiple forms of pectin methylesterase has been found in several products such as kiwis (Giovane *et al.*, 1990), apples (Castaldo *et al.*, 1989) and mandarin orange (Rillo *et al.*, 1991).

Versteg *et al.* (1980), studying and characterizing the pectin methylesterase in oranges, reported that this product contains at least three forms of PME. They observed that form 3 (PME 3), although the least abundant, was the most thermoresistant, and likely responsible for cloud loss in citrus fruit juices.

This hypothesis, however, does not seem to apply to the tomato. There are limited data in the literature describing multiple forms of PME (Pressey & Avants, 1972; Rexova-Benkova *et al.*, 1977). Unfortunately, the data regarding thermoresistance to heat in the different multiple forms have never been reported. It is therefore likely that the biphasic trend of PME thermoresistance (decimal reduction time versus temperature) may simply be due, as in the velocity of microorganism death (Casolari, 1988, 1991), to the velocity of PME thermal inactivation, or more generally the enzymes decrease with the drop in enzymatic concentration when treated thermically. It is more difficult to inactivate the biological unit (whether enzymes or microorganisms) as their numbers drop.

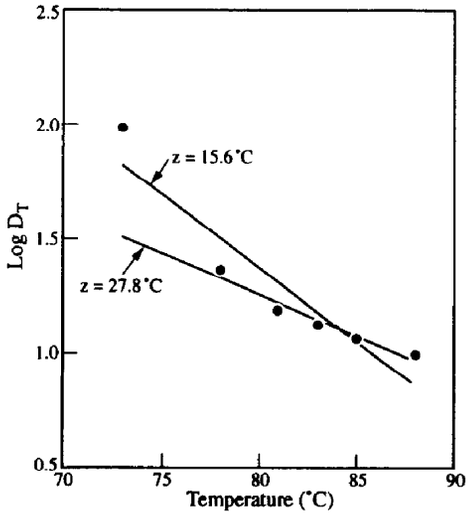

Fig. 2. Dependence of the decimal reduction time (D_T) for 'Rancho' variety as a function of temperature.

Table 2. *z* values for different tomato varieties

Tomato cultivars	*z* values (°C) calculated in the temperature range 73–88°C	*z* values (°C) calculated in the temperature range 78–88°C
Monterosso	15·6	31·2
Olter	21·0	34·6
Rancho	15·6	27·8
Spazio 1999	18·1	26·2
Zenith	18·5	27·3

Table 2 reports the *z* values in °C, for all the strains studied. To calculate the thermal treatment time needed to inactivate PME in tomato juice, the only data available are those extrapolated from the 78–88°C temperature range, that is to say, in the region of maximal thermal resistance. To evaluate *F*, *D* and *z* parameters so that the thermal treatment time to inactivate PME could be calculated, we assumed, as reported for citrus fruit juices (Rothschild *et al.*, 1975; Holland *et al.*, 1976), that tomato juice may be commercially stable (no cloud loss from PME) when PME activity drops to at least 10^{-4} U PME/ml of product.

Table 3 reports the *F*, *D* and *z* values for the single varieties examined. In Table 3 the decimal reduction times at any temperature may be calculated using log $D_T = \log D_{Tr} - (T - T_r)/z$, where T_r is the reference temperature defined as the temperature where 1 min ($F = 1$) is enough to inactivate the enzyme (PME activity $< 10^{-4}$ U PME/ml of tomato).

Table 3. *F*, *D*, *z* values of PME and *F/D* value for process calculations

Tomato cultivars	D_{Tr} (min)	*z* (°C)	T_r (°C)	*F/D*
Monterosso	0·17	31·2	81·0	5·6
Olter	0·18	34·6	85·4	5·5
Rancho	0·19	27·8	86·4	5·2
Spazio 1999	0·18	26·2	84·7	5·5
Zenith	0·17	27·3	85·2	5·6
Statistical parameters				
Min.	0·17	26·2	81·0	5·2
Mean	0·18	29·4	84·5	5·5
Max.	0·19	34·6	86·4	5·6
Standard deviation	0·01	3·4	2·1	0·2

Here the T_r reference temperature becomes an index of enzyme thermoresistance in the different varieties examined. Among the different varieties, the 'Monterosso' stands out (T_r = 81°C) as the least thermoresistant of those studied, and may be a good choice in industry for juices and pastes, while the 'Rancho' is the most thermoresistant (T_r = 86·4) of those studied.

CONCLUSIONS

The results seem to exclude that cloud loss in some tomato products such as a tomato juice and sauce may be linked to high PME thermoresistance. In fact, the *F/D* values for all the examined cultivars was much lower than those normally required for microbiological stabilization of these products. The different cultivars examined showed, however, a significant difference in PME activity and limited variation in thermoresistance. None the less, for each cultivar under study, a classic biphasic pattern of thermoresistance was seen (log D_T versus temperature); this featured a significant increase in the *z* parameter at high thermal treatment temperatures. The latter finding indicates that to determine correctly the *F*, *D* and *z* parameters, the heat inactivation kinetics need to be conducted at the highest possible temperature or roughly at the temperature usually used to stabilize these products (~95°C).

REFERENCES

Casolari, A. (1988). Microbial death. In *Physiological Models in Microbiology, Vol. II.* CRC Press Inc., FL.

Casolari, A. (1991). Letture di microbiologia — Inattivazione dei microorganismi. pp 77–81 (Windytower eds).

Castaldo, D., Quagliuolo, L., Servillo, L., Balestrieri, C. & Giovane, A. (1989). Isolation and characterization of pectin methylesterase from apple fruit. *J. Food Sci.*, **54**(3), 653–5 and 673.

Giovane, A., Qualiuolo, L., Castaldo, D., Servillo, L. & Balestrieri, C. (1990). Pectin methylesterase from *Actinidia chinensis* fruits. *Phytochem.*, **29**, 2821–3.

Holland, R. R., Reder, S. K. & Pritchett, D. E. (1976). An accelerated test for residual cloud reducing enzyme activity in citrus juices. *J. Food Sci.*, **41**, 812.

Joslyn, M. A. & Pilnik, W. (1961). Enzymes and enzyme activity. In *The Orange, its Biochemistry and Physiology*, ed. W. B. Sinclair. University of California Press, Berkeley, CA.

Koch, J. L. & Nevins, D. J. (1989). Tomato fruit cell wall: I. Use of purified tomato polygalacturonase and pectin methylesterase to identify developmental changes in pectins. *Plant Physiol.*, **91**, 816–22.

Krop, J. J. P. & Pilnik, W. (1974). Effect of pectic acid and bivalent cations on cloud loss of citrus juice. *Lebensm. Wiss. U. Technol.*, **7**, 62–66.

Nari, G., Noat, G. & Ricard, J. (1991). Pectin methylesterase, metal ions and plant cell-wall extension—Hydrolysis of pectin by plant cell-wall pectin methylesterase. *Biochem. J.*, **279**, 343–50.

Pressey, R. & Avants, J. K. (1972). *Phytochemistry*, **11**, 3139.

Rexova-Benkova, L., Markovic, O. & Foglietti, M. (1977). *J. Coll. Czech. Chem Commun.*, **42**, 1736.

Rillo, L., Castaldo, D., Giovane, A., Servillo, L., Balestrieri, C. & Quagliuolo, L. (1992). Purification and properties of pectin methylesterase from mandarin orange fruit. *J. Agric. Food Chem.*, **40**, 591–3.

Rothschild, G., Vanvliet, C. & Karsenty, A. (1975). Pasteurization conditions for juice and comminuted products of israeli citrus fruits. *J. Food Technol.*, **10**, 29.

Stumbo, C. R. (1973). *Thermobacteriology in Food Processing* (2nd edn). Academic Press, New York.

Versteg, C., Rombouts, F. M., Spansen, C. H. & Pilnik, W. (1980). *J. Food Sci.*, **45**, 969.