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Kinetics of thermal inactivation of tomato lipoxygenase

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Abstract

The kinetics of thermal inactivation of tomato lipoxygenase were studied. Tomato dices and a non-purified enzymatic extract were treated at different time–temperature combinations in the range of 0–150 min and 80–98 °C and the thermodynamic (activation energy) and kinetic (D_T and z) parameters for lipoxygenase inactivation were calculated. The kinetic behaviour of lipoxygenase from the tomato samples indicated the presence of two different isoenzymes having different thermal stabilities. Furthermore, the results showed that the isoenzymes were more heat-resistant than expected on the basis of literature reports and that the determined D_T and z values for lipoxygenase were orders-of-magnitude larger than those for microrganisms. This suggested that high temperature-short time treatments cannot be sufficient to achieve complete inactivation of tomato lipoxygenase.

Keywords: Lipoxygenase; Thermal treatments; Tomato; Kinetics; Enzyme inactivation

1. Introduction

As is known, for a given increase in temperature, the rate of destruction of microrganisms increases faster than the rate of destruction of nutrients and sensory components, due to their different activation energies (Labuza & Taoukis, 1990). Therefore, thermal processes are generally conducted under conditions which lead to microbial destruction with limited loss of nutritive and sensory attributes. Although most enzymes have thermal parameters (D_T and z) similar to those of microrganisms and are therefore inactivated during common pasteurisation and sterilisation processes, some enzymes may not be destroyed, because they are very heat-resistant (Williams, Lim, Chen, Pangborn, & Whitaker, 1986). This is particularly the case in acid foods, which are subjected to thermal treatments that are carried out at relatively low temperatures and/or for short times, which are sufficient for destroying spoilage microrganisms. Consequently, residual enzyme activities may cause changes to a product's quality attributes during storage (David, 1996).

Blanching is a commonly used pre-treatment that is normally carried out between the preparation of the raw material and later operations (heat sterilisation, dehydration or freezing) (Fellows, 2000). One of the main functions of blanching is to destroy enzymatic activities in vegetable products, prior to further processing, to minimise the possibility of quality deterioration during storage. Application of a correct blanching treatment is based on knowledge of some process and product variables, such as a product nature, size and shape, heating modality (mechanism of heat transfer, heating and cooling rates and product thermal coefficient), as well as knowledge of the most-heat resistant enzyme and its inactivation parameters (Anthon & Barret, 2002; Fellows, 2000; Martens, Scheerlinck, De Belie, & De Baerdemaeker, 2001). Generally, peroxidase is considered to be the indicator enzyme for process

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optimisation because of its high thermal stability. Consequently, the absence of residual activity of this enzyme would indicate that other less-resistant enzymes are also destroyed (Williams et al., 1986). Although this is also the case in tomato, recent findings have shown that lipoxygenase activity may be better correlated with quality changes (colour-loss and off-flavour development) of tomato derivatives (Barrett & Theerakulkait, 1995; Barret, Garcia, Russel, Ramirez, & Shirazi, 2000; Cabibel & Nicolas, 1992; Williams et al., 1986). As is known, tomato is one of the most widely consumed vegetables in the world, either as processed product (e.g., tomato juice, paste, puree, sauce and ketchup) or as an ingredient in food formulations, such as ready-to-eat products, frozen formulas (Rao, Waseem, & Agarwal, 1998). Nevertheless, published data on the thermal stability of lipoxygenase in tomato is very scarce (Anthon & Barret, 2003). Paradoxically, to our knowledge, a large number of literature reports deal with the effects of non-conventional processing (pulsed electric fields, hydrostatic pressure) on the inactivation kinetics of tomato lipoxygenase (Denys, van Loey, & Hendrickx, 2000; Min, Min, & Zhang, 2003b; Min, Jin, & Zhang, 2003a; Shook, Shellhammer, & Schwartz, 2001; Tangwongchai, Ledward, & Ames, 2000).

The aim of this investigation was to determine the kinetic parameters for lipoxygenase inactivation of tomato products. In particular, the inactivation kinetics were measured on diced tomato and on a non-purified enzymatic extract. Such tomato products, characterised by different matrix complexities and presumably by different mechanisms and rates of heat transfer, were chosen instead of purified commercial preparations, in order to obtain information relevant to tomato processing at the industrial level.

2. Materials and methods

2.1. Sample preparation

Ripe, fresh tomatoes (*Lycopersicon esculentum* cv. pyriforme) were purchased at a local market. They were washed and then diced into circa 0.5 cm cubes or subjected to an extraction procedure in order to obtain a non-purified enzymatic preparation. The extraction methodology described by Bonnet and Crouzet (1977) was followed. Aliquots of 20 g of tomato were blended for 3 min at 4 °C in a Polytron (Kinematica, Switzerland) with 20 ml Tris–HCl buffer (0.5 M, pH 8.0) containing 1% (w/v) ascorbic acid and 1% (w/v) EDTA. The homogenate was centrifuged at 3500*g* for 15 min at 20 °C. A quantity of 2% (v/v) $CaCl_2$ (1 M) was added to the supernatant in order to allow pectins to precipitate. After 2 h the suspension was centrifuged again at 3500*g* for 20 min at 20 °C.

2.2. Thermal treatments

Aliquots of ≈20 g of tomato samples were placed in glass cylinders (2 cm diameter, 20 cm height, 1.0 mm thickness), which were then hermetically sealed with butyl septa and metallic caps. Heat treatments were carried out in a thermostatted water bath equipped with a stirring device. The capacity of the water bath was sufficient to prevent any recordable drop in the temperature during the experiment. Samples were heated at different time–temperature combinations in the ranges of 80–98 °C and 0–150 min. After heating, the samples were rapidly cooled by running cold water until they reached a temperature of 30 ± 3 °C. During the thermal treatment, temperature was measured (with an accuracy of ±0.2 °C) by a copper/constant thermocouple probe (type T thin thermocouple, 1.2 mm diameter, embedded in a stainless steel hypodermic needle, Ellab, Denmark), whose tip was placed at the slowest heating point of the tomato sample. A thermocouple probe was also used in order to measure the water temperature. Time and temperature data were acquired by means of a data logger (Keithley, 2700 Multimeter, Cleveland, Ohio, USA) connected with a computer,either during the heating or the cooling processes, and the thermal effects (i.e., *F*-values referred to 100 °C and a *z* value of 10 °C) automatically calculated. Two separate heat penetration runs were carried out, with two replicate cases in each run, thus four results in close agreement were obtained. An unheated sample was taken as a reference.

2.3. Analytical determinations

2.3.1. Lipoxygenase activity

Lipoxygenase activity was assayed by the method of Bonnet and Crouzet (1977) based on absorption at 234 nm of the conjugated dienes formed when linoleic acid (used as substrate) was oxidised in the presence of lipoxygenase. The substrate consisted of 10 μl of linoleic acid, 4 ml of H_2O, 1 ml of 0.1 N NaOH and 5 μl of Tween 20. The mixture was shaken and diluted to 25 ml with water. Each test contained 2.7 ml of phosphate buffer (0.2 M, pH 6.5), 0.3 ml of the substrate and 50 μl of enzyme extract (prepared as described previously) in a 1 cm cell. After incubation for 10 min at 30 °C, the reaction was started by addition of the enzyme and the increase in absorbance at 234 nm (A_{234}) was followed with a Varian DMS 80 spectrophotometer (Varian Technotron Pty. Ltd., Mulgrave, Australia). In order to overcome interference due to absorbance, at this wavelength, of other tomato components, 1 ml of 1 N NaOH was added to the system after 8 min of reaction (Anthon & Barret, 2003). The addition of NaOH caused enzyme denaturation, as well as a shift of the acid-base equilibrium of linoleic acid to its salt form; thus a clear solution was obtained. The residual activity

was calculated as the percentage ratio between the activity at time t and that of the unheated sample. One unit of activity was defined as the increase of 0.10 A_{234} min^{-1} under the conditions previously described. Protein content of enzymatic preparation was measured by the Bradford method (Bradford, 1976), using bovine serum albumin as a standard and measuring absorbance at 600 nm. Lipoxygenase specific activity was expressed in nmoles of conjugated dienes formed under the assay conditions per second (nkat) per 1 mg of enzyme protein per g of tomato dry matter.

2.3.2. Texture

The texture of tomato was evaluated by measuring the penetration force using a puncture test (Thompson, Fleming, Hamann, & Monroe, 1982). The maximum force value is related to the firmness of tomato. An Instron texturometer (Instron Series 4200, Instron Ltd, UK), equipped with a cylindrical puncture probe, 3.0 mm in diameter, was used. The texture was measured by puncturing the surface of the whole samples at a constant speed of 50 mm/min.

2.3.3. Colour

Colour analyses were carried out on tomato purees using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The instrument was standardised against a white tile before measurements. Colour was expressed in L^*, a^* and b^* Hunter scale parameters and a^* and b^* were used to compute hue angle ($\tan^{-1} b^*/a^*$) (Clydesdale, 1978).

2.3.4. pH

The pH was measured at 25 °C using a pH meter (Hanna Instruments, model 8417, Milano, Italy), equipped with a combination of glass electrode and a temperature probe.

2.3.5. Soluble solids

Soluble solids were measured at 25 °C using a refractometer (Unirefrax, Bertuzzi, Milano, Italy).

2.3.6. Total solids content

Total solids content was determined by the gravimetric method according to AOAC methods (AOAC, 1995).

2.4. Calculations

2.4.1. Enzymatic activity

The inactivation kinetics of tomato lipoxygenase were analysed by the two-fraction model, based on the assumption that there were heat-labile and heat-resistant isoenzymes whose thermal destruction obeys pseudo first order kinetics (Ling & Lund, 1978). Thermal inactivation curves from enzyme systems consisting of heat-labile and heat-resistant fractions should behave according to Eq. (1)

$$\%\text{activity} = \frac{k_l C_l e^{-k_l t} + k_r C_r e^{-k_r t}}{k_l C_l + k_r C_r}, \quad (1)$$

where C_l and C_r are the concentrations of heat-labile and heat-resistant fractions, respectively, and k_l and k_r are the rate constant for heat-labile and heat-resistant isoenzymes, respectively.

For long heating time, $e^{-k_l t}$ will be close to zero and Eq. (1) becomes

$$\%\text{activity} = \frac{k_r C_r}{k_l C_l + k_r C_r e^{-k_r t}}. \quad (2)$$

For short thermal treatment, Eq. (1) can be and approximated as

$$\%\text{activity} = \frac{k_l C_l}{k_l C_l + k_r C_r e^{-k_l t}} + \frac{k_r C_r}{k_l C_l + k_r C_r}. \quad (3)$$

By applying the limiting conditions at long and short thermal processing times, the thermodynamic parameters of the heat-labile and heat-resistant isoenzymes can be determined from thermal destruction curves (Ling & Lund, 1978).

The reaction rate constants for heat-resistant and heat-labile fractions were determined by using the Microsoft Excel 97 software programme.

2.4.2. Enzyme inactivation parameters

Enzyme inactivation was expressed in terms of thermal stability parameters, i.e. D_T and z values. The D_T, or decimal reduction time value, is defined as the time required to inactivate 90% of the original enzyme activity at a constant temperature, and it is obtained from plots of log (enzyme activity) as a function of time. The z value is the temperature needed to reduce the D_T value by one log-unit, and it is obtained from the plot of log D_T against temperature.

D_T and z values for both heat-labile and heat-resistant enzymatic fractions (D_{Tl} and z_l, D_{Tr} and z_r, respectively) were calculated.

Activation energies for the thermal inactivation of lipoxygenase were calculated using the Arrhenius equation.

2.5. Data analysis

The results reported here are the average of at least three measurements, and the coefficients of variation, expressed as the percentage ratio between the standard deviation (SD) and the mean values, were below 10 for colour and texture, 2 for total and soluble solids content and 15 for enzyme activity and protein concentration. One-way analysis of variance was determined using the Tukey–Krammer test. Differences between means were considered to be significantly different at $P < 0.05$.

3. Results and discussion

As already pointed out, optimisation of the thermal treatment for enzymatic inactivation presupposes knowledge of product and process variables. Table 1 shows some chemical and physicochemical characteristics (colour, soluble and total solids, texture and pH values), as well as the lipoxygenase specific activity of the tomato fruits used in the present investigation. All these values are in agreement with the data reported in the literature for tomato (Cabibel & Nicolas, 1992; Gould, 1992; Shook et al., 2001).

As exemplified in Fig. 1, which shows the experimental temperature profiles of tomato samples heated at 90 °C for increasing lengths of time, the come-up time was approximately equal to 7 and 3 min, respectively, for the tomato dices and the non-purified enzyme extract at all the temperatures considered, while the come-down time was practically instantaneous. Thus, as expected, the come-up time depended on the matrix complexity, with longer heating required for the diced tomato to reach the set temperature.

Figs. 2 and 3 show lipoxygenase residual activities of tomato dices and extracts as a function of heating time at three different temperatures. As expected, enzymatic activity decreased as the heating time increased. In all samples, and especially in those heated at the highest temperatures, a sharp decrease in lipoxygenase activity was observed in the first few minutes of heating, followed by a slower decrease in activity for prolonged heating times. This suggests the presence of at least two isoenzymes having different thermal stabilities. Resistant and labile lipoxygenase isoenzymes have already been observed in many vegetable products (potato, wheat germ, green beans, peas), as well as in tomato (Anthon & Barret, 2003; Bhirud & Sosulski, 1993; Busto et al., 1999; Indrawati, Ludikhuyze, VanLoey, & Hendrickx, 1999; Nicolas, Autran, & Drapron, 1982; Park, Kim, & Lee, 1988; Smith, Linforth, & Tucker, 1997; Williams et al., 1986). Such biphasic behaviour of inactivation kinetics of labile (k_l) and resistant (k_r) isoenzymes was analysed by means of the two-fraction model (Eqs. (2) and (3)) proposed by Ling and Lund (1978). The estimated rate constants of the heat-labile and heat-resistant fractions, obtained by treatment of experimental data, are presented in Table 2. Table 2 also shows activation energies for thermal destruction of the labile and resistant isoenzymes of tomato dices and enzymatic extract obtained from the slopes of the Arrhenius plots (not shown). These activation energies are

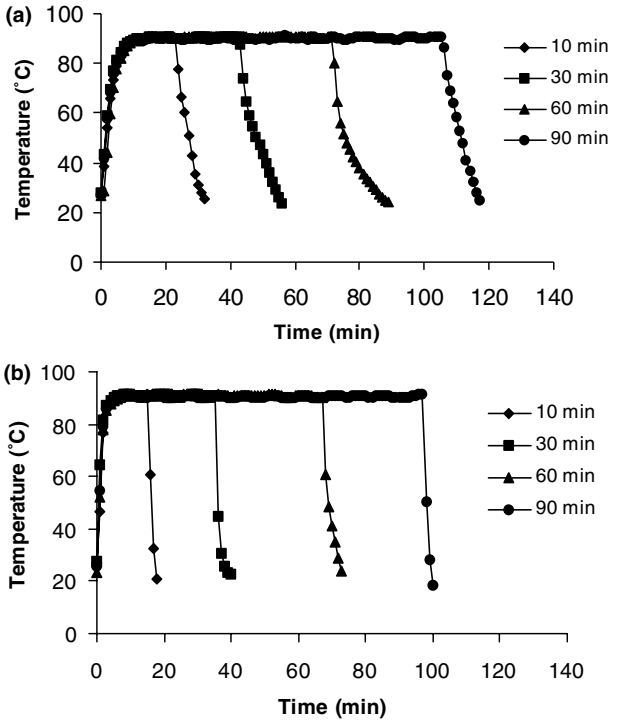

Fig. 1. Heat penetration curves at 90 °C of tomato dices (a) and of a non-purified lipoxygenase extract (b).

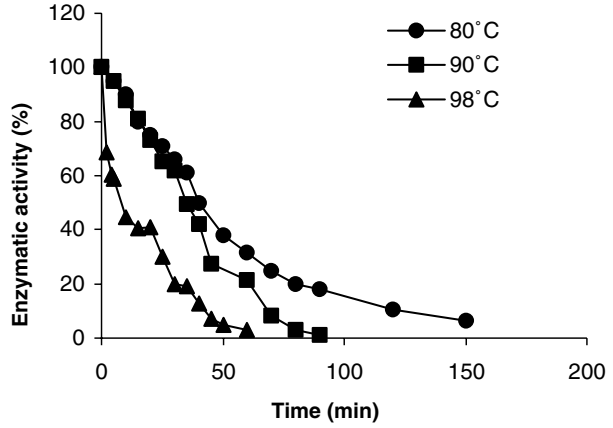

Fig. 2. Thermal inactivation curves for lipoxygenase from tomato dices.

Table 1
Quality parameters and lipoxygenase specific activity of the tomato sample under investigation

Colour		Soluble solids (°Bx)	Total solids (%)	Texture (N)	pH	Lipoxygenase specific activity (nkat mg^{-1} enzyme protein g_{ss}^{-1})
L^*	Hue angle ($\tan^{-1} b^*/a^*$)					
31.9 ± 0.8	28.5 ± 3.0	5.0 ± 0.5	7.9 ± 0.15	6.29 ± 0.60	4.3 ± 0.02	0.169 ± 0.022

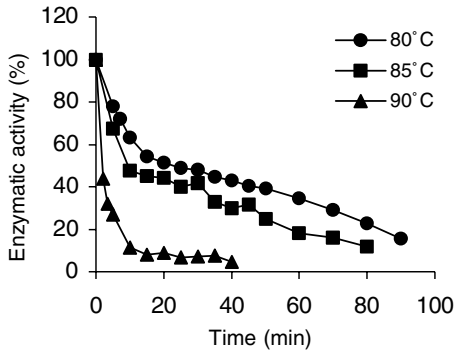

Fig. 3. Thermal inactivation curves for a non-purified lipoxygenase extract from tomato.

comparable to those reported for lipoxygenase from green beans, peas and soybean (Garrote, Silva, & Bertone, 2001; Güneş & Bayindirli, 1993; Henderson, Blank, & Sustackova, 1991; Indrawati et al., 1999; Svensson & Eriksson, 1974), but lower than those reported for potatoes and tomatoes (Anthon & Barret, 2003; Kermasha, Bisakowski, Ramaswamy, & Van de Voort, 1993; Østergaard Alsøe & Adler-Nissen, 1988; Park et al., 1988).

Since the range of inactivation temperatures investigated was quite narrow (less than 20 °C), the more commonly used D_T and z values for the enzyme systems were also estimated (Table 3). At a constant temperature, only slight differences between the decimal reduction times (D_{Tr}) of the heat-resistant fractions of the two tomato matrices were observed. This suggests that the matrix complexity of the product did not greatly influence the rate of lipoxygenase inactivation. On the other hand, the integrity of the vegetable matrix greatly affected the D_{Tl} of the heat-labile isoenzymes. In particular, the thermal resistance of the heat-labile lipoxygenase fraction decreased with the increasing of breakdown degree of tomato tissue. Similarly, Williams et al. (1986) found that lipoxygenase was more stable in whole peas than in the homogenised derivative. In addition, from Table 3 it can be observed that, as expected, the higher the structure complexity the greater were the z_r and z_l values.

Table 3
Thermal inactivation parameters of lipoxygenase of tomato dices and a non-purified lipoxygenase extract

Sample	Inactivation temperature (°C)	D_{Tr}[a] (min)	D_{Tl}[a] (min)	z_r[b] (°C)	z_l[b] (°C)
Tomato dices	80	143	86.9		
	90	48.8	25.6		
	98	34.5	8.9	28.7	18.2
Tomato extract	80	115	12.2		
	85	83.3	9.3		
	90	37.3	3.0	20.6	16.5

All measurements were replicated at least three times. Experimental data were reproducible with less than 15% variance.

[a] D_{Tr} and D_{Tl}: decimal reduction times of the heat-resistant and heat-labile fractions, respectively.

[b] z_r and z_l of the heat-resistant and heat labile-fractions, respectively.

In summary, these results show a high thermal resistance of tomato lipoxygenase, suggesting that blanching treatments generally applied (usually a few minutes or seconds, respectively, in boiling water or vapour steam) are probably not sufficient to achieve complete inactivation. However, these results are in disagreement with the data reported by Anthon and Barret (2003), indicating that both labile and resistant forms of tomato lipoxygenase are rapidly inactivated at 60 °C. Such differences in kinetic data may be attributed to differences in the raw material, such as exact variety, maturity, and enzyme concentration, as well as in the heating modalities (Martens et al., 2001; Williams et al., 1986). It is a matter of fact that different tomato varieties with different enzyme activities were used in Anthon and Barret's and our experiments. Moreover, in the former trials, heating was performed in capillary tubes, which lead to instant heat transfer but are not representative of industrial processing.

For process purposes, great advantage can be taken by representing the spatial relationship of equivalent iso-thermal treatments. Figs. 4 and 5 show the time–temperature combinations leading to a similar thermal effect (iso-F value), necessary to cause the same number

Table 2
Rate constants and activation energies for destruction of lipoxygenase activity of tomato dices and a non-purified lipoxygenase extract

Sample	Inactivation temperature (°C)	k_r[a] (min^{-1})	k_l[a] (min^{-1})	Ea_r[b] (kJ/mol)	Ea_l[b] (kJ/mol)
Tomato dices	80	0.45	0.03		
	90	2.02	0.09		
	98	2.21	0.26	99.7	137
Tomato extract	80	1.13	0.19		
	85	1.27	0.25		
	90	2.00	0.76	60.8	147

All measurements were replicated at least three times. Experimental data were reproducible with less than 15% variance.

[a] k_r and k_l: rate constants for thermal inactivation of the heat-resistant and heat-labile fractions, respectively.

[b] Ea_r and Ea_l: activation energies of the heat-resistant and heat labile-fractions, respectively.

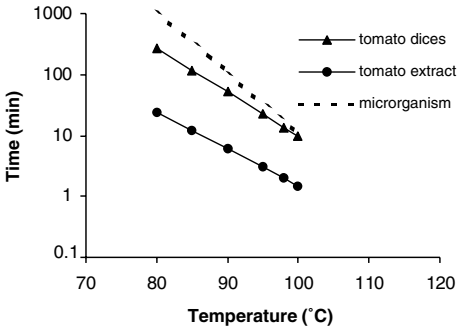

Fig. 4. Equivalent time–temperature conditions towards the inactivation of the heat-labile lipoxygenase fractions and the destruction of *C. pasteurianum* and *B. coagulans* spores in tomato products.

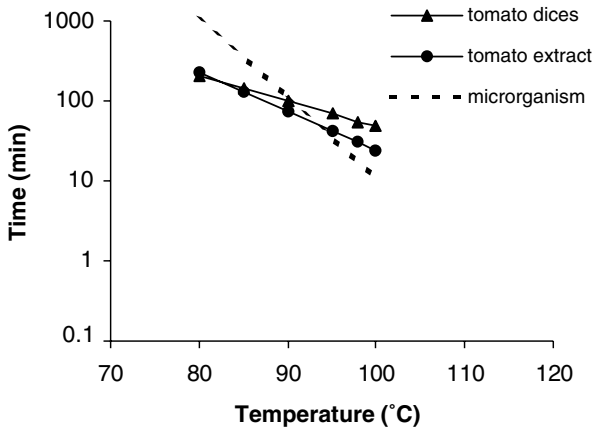

Fig. 5. Equivalent time–temperature conditions towards the inactivation of the heat-resistant lipoxygenase fractions and the destruction of *C. pasteurianum* and *B. coagulans* spores in tomato products.

of decimal reductions ($n = 2$) of lipoxygenase activity of the tomato dices and extract. The considered iso-F values were calculated by using the following equation (Ramaswamy & Chen, 2002):

$$\frac{F_{T_1}}{F_{T_2}} = 10^{(T_2 - T_1)/z}, \tag{4}$$

where T_1 is the actual temperature and T_2 is a reference temperature, which was arbitrarily chosen equal to 90 °C; the experimentally determined z values were used.

As is known, each line of Figs. 4 and 5 identifies the equivalent time–temperature conditions towards enzymatic inactivation. Therefore, time–temperature combinations which lie below the iso-lines do not lead to enzymatic inactivation; on the contrary, those above the iso-lines are adequate to lead to destruction of lipoxygenase activity. Except for the heat-resistant lipoxygenase from both tomato dices and extract that had approximately the same F-values at temperatures below 90 °C, in the considered range of time–temperature combinations, the higher the complexity of the vegetable matrix the higher were the F-values at a constant temperature. For instance, considering that the z-values of the heat-labile lipoxygenase from the diced tomato and extract were, respectively, equal to 18.2 and 16.5 °C, about 1.5 min at 100 °C will be sufficient to lead to destruction of the lipoxygenase extract, while a time more than 6 times longer is needed to inactivate lipoxygenase of the diced tomato. Also, in Figs. 4 and 5, the time–temperature data for lipoxygenase inactivation were superimposed on the graph to plot the thermal death-time curve for *Clostridium pasteurianum* and *Bacillus coagulans* spores, which are the most important spoilage-causing microrganisms in tomato products of pH 4.5 or less (Leoni, 1993). The thermal inactivation parameters for these bacteria (i.e., F value equivalent to 11 min at 100 °C with a 10 °C z-value) are generally used at the industrial level for the stabilisation of tomato derivatives. It can be observed that the thermal treatments (from 80 to 100 °C), having the sterilising effect required to destroy spoilage microrganisms, were also adequate for the inactivation of the labile lipoxygenase fraction. On the other hand, the heat-resistant isoenzyme can be inactivated by sterilising at temperatures below 95 °C, whereas processing to the same F-value for microbial destruction, at higher temperatures, did not lead to inactivation of either the dices or the extract. In fact, as both the D_T values for the tomato samples and their dependence on temperature (z values) are orders-of-magnitude larger than those for microrganisms, high temperature-short time treatments (HTST) led to limited inhibition of lipoxygenase activity. This result is in agreement with the observation that peroxidase may survive the HTST process due to its high D_T and z values (Adams, 1978; Herson, 1991).

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