Kinetics for Isobaric−Isothermal Degradation of L-Ascorbic Acid

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A study of the thermal degradation of L-ascorbic acid in different buffer systems (pH 4, 7, and 8) and in real products (juice from squeezed oranges and tomatoes) revealed that L-ascorbic acid is most thermosensitive in real products. These products were selected to investigate in detail the degradation kinetics of L-ascorbic acid by heat (120−150 °C) as well as by combined pressure−temperature treatment (8.5 kbar and 65−80 °C). In both cases the L-ascorbic acid degradation was confirmed to be a first-order reaction. The *z* values of thermal destruction of L-ascorbic acid in squeezed oranges and tomatoes were respectively 27.15 and 30.15 °C and were shown to be similar for pressure−temperature degradation.

Keywords: *L-Ascorbic acid; thermal stability; pressure stability; kinetics; pH*

INTRODUCTION

Consumers demand foods of high quality, with particular regard to their nutritional aspects, rather than overheated foods. There are many indications that high-pressure treatment, a new promising technology, can meet these demands. As it only affects noncovalent bonds, i.e., hydrogen bonds, ionic bonds, and hydrophobic bonds (Hayashi et al., 1989; Knorr, 1993), high pressure causes inactivation of microorganisms and denaturation of several enzymes but leaves most quality carriers, such as taste, flavor, color, and vitamin/nutrient content intact. In contrast to heat sterilization, this does not lead to a loss in vitamins or to off-flavor.

Vitamin C (L-ascorbic acid) is a typical heat sensitive nutrient (Saguy et al., 1978). Therefore its retention is often regarded as a significant marker of overall nutrient recovery (Lee et al., 1976; Jung et al., 1995; Hurt, 1979). Moreover the evidence that the antioxidant nutrients, such as vitamin C or L-ascorbic acid, may play a much more important role in our health and well being is growing rapidly (Johnson, 1995). Vitamin C is hypothesized to prevent cancer by inhibiting the formation of *N*-nitroso compounds in the stomach and by stimulation of the immune system (Byers and Perry, 1992).

There are numerous reports in the literature about the instability of ascorbic acid during storage (Lee et al., 1977; Robertson and Samaniego, 1986; Lee and Nagy, 1988) and thermal processing (Howard et al., 1994; Lathrop and Leung, 1980; Rao et al., 1981; Laing et al., 1978), and many articles, discussing high-pressure-related topics, mention the pressure resistance of ascorbic acid (Cheftel, 1991; Ogawa et al., 1992; Eshtiaghi and Knorr, 1993; Donsi et al., 1996; Quaglia et al., 1996; Yen and Lin, 1996). However, only limited kinetic data are available on ascorbic acid losses during thermal and combined pressure−temperature treatments.

As kinetic information is indispensable with regard to optimization of this new technology, the objective of this study was to investigate the degradation of L-ascorbic acid by heat and/or pressure on a kinetic basis. Not only buffered model systems but also natural products were taken into consideration. For natural products, we decided to focus on tomatoes and oranges. These products are of particular interest for the application of high pressure since the natural low pH is an additional inhibitory factor to pathogenic food-related microorganisms (Parish et al., 1994). Moreover, a large amount of L-ascorbic acid is naturally present in these products (Belitz and Grosch, 1987).

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MATERIALS AND METHODS

L-Ascorbic Acid and Media. *Model System.* L-Ascorbic acid (pro analysis) was purchased as a dry powder from Merck (Overijse, Belgium) and dissolved in different environments in a concentration of 2.5 mg/mL. To study the influence of pH on the degradation of L-ascorbic acid the following buffers were used: *m*-phosphoric acid buffer (1.5% (w/v)), pH 4, phosphate buffer 0.1 M, pH 7, and phosphate buffer 0.1 M, pH 8.

Natural Products. To investigate the degradation of L-ascorbic acid in natural products, which are of importance for the high-pressure technology, tomatoes and oranges bought in a local supermarket were used. After the products were squeezed, the juice was filtered through a Whatman No. 5 filter paper to remove excess pulpy material and juice sacs. Although the amount of L-ascorbic acid naturally present in tomatoes and oranges (respectively 24.4 mg/100 g and 50 mg/100 g (Belitz and Grosch, 1987)) is relatively high, an additional amount of commercial L-ascorbic acid was added to the filtrate. The amount of L-ascorbic acid naturally present in tomatoes and oranges was experimentally determined as 0.1 and 0.5 mg/mL, respectively. To obtain similar initial concentrations of L-ascorbic acid in the different buffer systems and both filtrates, 2 mg/mL of commercial L-ascorbic acid was added to the orange filtrate (pH 3.5) whereas 2.4 mg/mL of commercial L-ascorbic acid was added to the tomato filtrate (pH 4.5). The pH values of the two tomato juices were comparable. We assumed that L-ascorbic acid, naturally present in these products, reacts in a similar way toward pressure and temperature as does added commercial L-ascorbic acid. The samples were heat- and/or pressure-treated immediately after sample preparation so that there was no L-ascorbic acid loss during storage.

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Determination of L-Ascorbic Acid Concentration. The concentration of L-ascorbic acid was measured spectrophotometrically. This colorimetric method is based on the reduction of the tetrazolium salt MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) by L-ascorbic acid in the presence of the electron carrier PMS (5-methylphenazinium methyl sulfate) at pH 3.5 to a formazan. The MTT−formazan is the measuring parameter and is determined by means of its absorbance in the visible range at 578 nm.

But as L-ascorbic acid is not the only reducing substance in the sample, a blank determination is necessary. In a blank determination, only the ascorbate fraction as part of all reducing substances present in the sample is oxidatively removed by L-ascorbic acid oxidase (AAO) in the presence of oxygen. The dehydroascorbate formed does not react with MTT−PMS.

For the determination of the concentration in squeezed tomatoes and oranges, no additional preparations (e.g. decolorization) were needed. Before the measurement of the concentration, the sample (model system and natural product) must be diluted sufficiently to yield a concentration of L-ascorbic acid between 0.01 and 0.20 g/L. All measurements were performed in darkness, according to procedure No. 409 677 of Boehringer (*Methods of Enzymatic Food Analysis*; Mannheim GmbH, 1986).

Determination of Oxygen Concentration. To determine the amount of oxygen in the juice sample before and after treatment, an oxygen meter (Strathkelvin Instruments model 781) was used.

Isothermal Treatment. Isothermal experiments were performed in an oil bath with temperature control. To ensure isothermal heating, the L-ascorbic acid solution was enclosed in capillary tubes (Hirschmann, 1.15 mm i.d., 150 mm length) using a vacuum pump. In this way, the amount of residual oxygen in the capillaries was negligible. After preset time intervals, the capillaries were withdrawn from the oil bath and immediately cooled in ice water. The concentration of L-ascorbic acid was measured after 10−120 min of storage in ice water. The temperature range studied varied from 120 to 150 °C.

Isobaric−Isothermal Treatment. To perform isobaric−isothermal treatments, laboratory pilot scale, multivessel high-pressure equipment (HPIU-10.000 serial no. 95/1994, Resato, Roden, The Netherlands) was used. The apparatus allows pressurization up to 10 kbar in combination with temperatures ranging from 20 to 100 °C. High pressure is generated using a pressure intensifier in the central pressure circuit. The pressure medium is a glycol−oil mixture (TR15, Resato). The temperature is controlled by a thermostated mantle which surrounds each vessel and which is connected to a cryostat. This apparatus is ideally suited for kinetic studies, since eight individual vessels (volume = 8 mL, diameter = 10 mm, length = 100 mm) can be subjected to the same pressure level and the same temperature level.

Isobaric−isothermal treatments were performed as follows: flexible microtubes (0.3 mL, Elkay, Overijse, Belgium) were filled with solution, thereby avoiding air bubbles. Part of the juice sample was subjected to nitrogen gas flush (to remove oxygen) before the tubes were filled. Afterward, the tubes were enclosed in the pressure vessels, which were equilibrated at a preset temperature. Initially, the valves of the pressure vessels were open so that pressurization of the central tubing led to pressurization of the individual vessels. After the pressure had reached the desired level, the vessels were isolated so that the pressure was maintained in the vessels until the valves were opened. As pressure buildup is associated with adiabatic heating, there was a temperature increase in the vessels, which depends on the speed of the pressure buildup (Weemaes et al., 1997). As soon as the temperature was again equilibrated at the preset temperature in the eight vessels, the time of the experiment was started. At this moment, each vessel was subjected to the same pressure and temperature levels and the first valve was opened. By regarding the concentration of the sample in this "reference" vessel as the initial concentration (C_0), i.e. the concentration at $t = 0$, the process could be considered as an isobaric−isothermal treatment. The other valves were opened after preset time intervals.

In all experiments the pressure buildup was slow so that the temperature increase was minimal. After pressure release the samples were immediately cooled in ice water. The concentration was measured after 10−120 min storage in ice water. The temperature range studied varied from 65 to 80 °C at a pressure of 8500 bar.

Data Analysis. First-order reactions can be described by the following formula:

$$\frac{dC}{dt} = -kC \tag{1}$$

Under isothermal (= constant temperature) and isothermal−isobaric (= constant temperature and constant pressure) conditions, the inactivation rate k is constant. The integration of eq 1 yields 2.

$$\ln \frac{C}{C_0} = -kt \tag{2}$$

The temperature dependence of k is given by the activation energy (E_a), as indicated in the Arrhenius relationship.

$$k = k_{ref} \exp\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \tag{3}$$

This relation is valid at atmospheric pressure, and we assume that this relation is also valid at elevated pressure.

In the area of food processing, it is common to characterize first-order reactions in terms of D and z values (thermal death time concept). The decimal reduction time (D value) is the time, at a given temperature and/or pressure, needed for a 90% reduction of the initial concentration. The relation between the D value and the more general inactivation rate constant (k) is given by eq 4.

$$D = \frac{\ln(10)}{k} \tag{4}$$

The temperature dependence of the D value is given by the z value. The z value equals the temperature increase necessary to obtain a 10-fold decrease of the D value. The z value is, in fact, an alternative to the activation energy.

Based on experimental data, k and D values were calculated from linear regression of the natural (ln) and ten-based (log) logarithms of the concentration retention versus processing time. The E_a and z values were estimated from linear regressions of ln(k) versus (1/T) and of log(D) versus T, respectively.

RESULTS AND DISCUSSION

Screening Study. A screening study was performed in order to determine the thermostability of L-ascorbic acid as a function of pH. Hereto, commercial L-ascorbic acid was dissolved in different buffers such as 0.1 M phosphate buffer, pH 7 and 8, and *m*-phosphoric acid, 1.5% (w/v), pH 4. Also natural products with low pH were taken into consideration because it is known that the kinetic data obtained from model buffer systems may not be applicable to food systems (Lathrop and Leung, 1980). As mentioned in the Introduction, we decided to focus on the natural products tomatoes and oranges because these products are of particular interest for the application of high pressure. For all environments studied, temperatures higher than 100 °C were needed to degrade L-ascorbic acid, and the breakdown could be accurately described by a first-order reaction. As the solubility of oxygen in water decreases with

Table 1. *D* Values for the Thermal Degradation of L-Ascorbic Acid in Different Environments

environment	D_{140} (min)
buffer, pH 4	176.8 ± 7.3[a]
buffer, pH 7	379.8 ± 15.6
buffer, pH 8	413.7 ± 19.1
squeezed oranges	47.93 ± 2.48
squeezed tomatoes	94.01 ± 4.93

[a] Standard error.

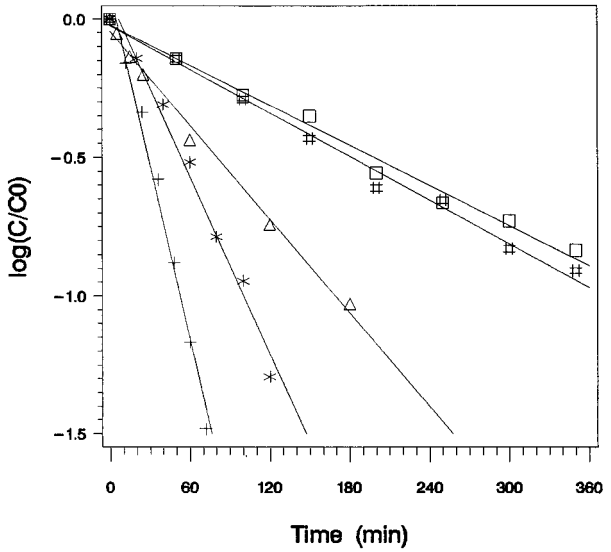

Figure 1. Thermal degradation of L-ascorbic acid at 140 °C in different environments; pH 8 (□), pH 7 (#), pH 4 (△), squeezed tomatoes (∗), and squeezed oranges (+).

increasing temperature and as the capillaries were filled under vacuum, we assume that L-ascorbic acid degraded mainly via an *anaerobic* pathway. Laing and coworkers (1978) also suggested a shift in reaction mechanism from oxidative to nonoxidative, when temperature exceeded 95 °C. Previous studies on anaerobic or aerobic degradation of ascorbic acid in canned peas (Lathrop and Leung, 1980) and tomato juice (Lee et al., 1977) also confirmed that the reactions followed first-order kinetics. Laing et al. (1978), on the contrary, found a zero-order reaction for the disappearance of ascorbic acid in model food systems. Lin and Agalloco (1979) reported that the first-order rate is valid only if the oxygen is present in abundance (for aerobic degradation) or if it is totally excluded (for anaerobic degradation). In instances where oxygen is present in a limited amount, second-order kinetics is followed, i.e., the reaction depends on both the oxygen and ascorbic acid concentrations. From a ranking of the buffer systems according to their *D* values (determined at a temperature of 140 °C), it appears that at higher pH values the degradation rate of L-ascorbic acid is retarded (see Table 1). The ranking of the systems can be seen more clearly in Figure 1, wherein L-ascorbic acid retention for each system is plotted versus heating time. Also Coker et al. (1993) found that increasing the pH from 0.5 to 11 reduces the *anaerobic* degradation of vitamin C whereas a maximum *aerobic* denaturation of vitamin C was found at higher pH values. The mechanism of anaerobic degradation of ascorbic acid has not been fully established. However, it is assumed that a direct cleavage of the 1,4-lactone bridge without prior oxidation to dehydroascorbic acid occurs. The opening of the lactone ring is favored by low pH values (pH 3−4)

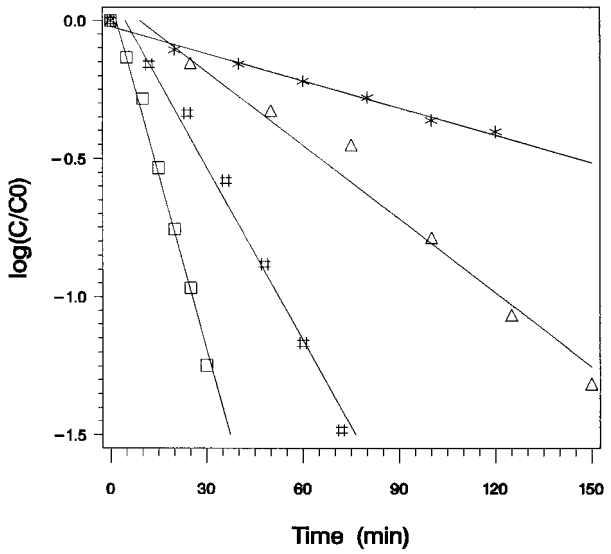

Figure 2. Thermal degradation kinetics of L-ascorbic acid in squeezed oranges at 120 (∗), 130 (△), 140 (#), and 150 (□) °C.

(Gregory, 1996). By comparing environments with similar pH values (buffer, pH 4; squeezed oranges, pH 3.5; squeezed tomatoes, pH 4.5) it can be stated that L-ascorbic acid is less thermostable in a natural environment. Heating orange juice during 48 min at 140 °C resulted in an 1 log unit reduction of the concentration of L-ascorbic acid, whereas in a buffer at pH 4, 177 min was needed to obtain a similar reduction rate at 140 °C. This can be explained by the fact that L-ascorbic acid is more sensitive to degradation in the presence of traces of metals (e.g., copper and iron), which are naturally present in juices. This is confirmed by a study of Lee et al. (1977), who reported that the rate of ascorbic acid degradation in canned tomato juice during storage increased with increased copper concentration (2, 6, and 10 ppm).

The most thermosensitive systems from this screening study, i.e., juice from freshly squeezed oranges and tomatoes, were selected to further investigate the thermal as well as the pressure−temperature degradation kinetics of L-ascorbic acid.

Isothermal Degradation Kinetics of L-Ascorbic Acid in Squeezed Oranges and Tomatoes. Isothermal degradation of L-ascorbic acid in squeezed oranges (2 mg/mL) and juice from two different varieties of tomatoes (2.4 mg/mL) was investigated in a temperature range from 120 to 150 °C. The log-linear decrease of the L-ascorbic acid concentration (dissolved in oranges) as a function of heating time is illustrated in Figure 2. The temperature dependence of the *D* value is given by the *z* value, which is visualized in Figure 3. The estimated kinetic parameters are summarized in Tables 2 (tomatoes) and 3 (oranges).

From Tables 2 and 3, it is clear that the rate of L-ascorbic acid degradation in each system increases with increasing temperature from 120 to 150 °C. Apparently, the sensitivity of L-ascorbic acid to heat depends not only on the type of product (oranges, tomatoes) but also on the origin of the sample. It can be stated that L-ascorbic acid is more sensitive to heat in oranges than in tomatoes. Also from literature data it appears that the instability of vitamin C is dependent on the source. Roig et al. (1995) reported an overall greater retention of ascorbic acid in processed orange juice than in lemon juice. Lee et al. (1976) even found

Table 2. *D*, *k*, *z*, and E_a Values for the Thermal Degradation of L-Ascorbic Acid (2.4 mg/mL) in Squeezed Tomatoes

	tomatoes, variety A			tomatoes, variety B		
T (°C)	D (min)	$k \times 10^{-1}$ (min^{-1})	r^2	D (min)	$k \times 10^{-1}$ (min^{-1})	r^2
120	469.26 ± 24.35[a]	0.049 ± 0.003	0.99	325.31 ± 20.14	0.071 ± 0.005	0.98
130	199.40 ± 11.56	0.115 ± 0.007	0.98	126.14 ± 4.93	0.183 ± 0.007	0.99
140	94.01 ± 4.93	0.245 ± 0.014	0.98	56.05 ± 2.73	0.411 ± 0.021	0.99
150	47.28 ± 2.46	0.487 ± 0.027	0.99	26.64 ± 1.55	0.864 ± 0.053	0.99

z (°C)	E_a (kcal/mol)	r^2	z (°C)	E_a (kcal/mol)	r^2
30.15 ± 1.02	25.2 ± 0.5	0.99	27.68 ± 1.04	27.5 ± 0.7	0.99

[a] Standard error.

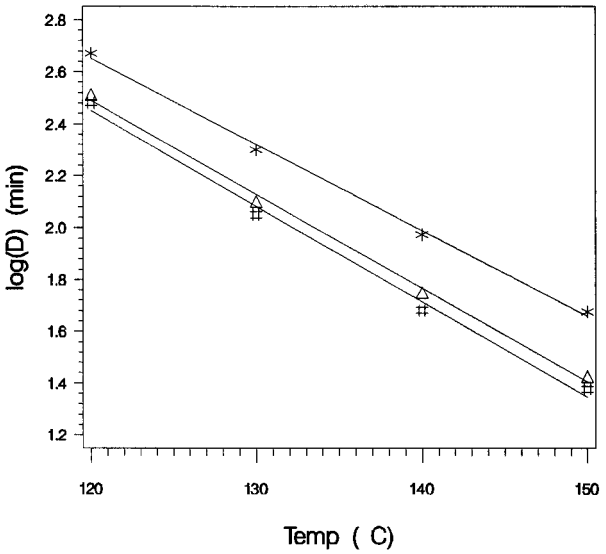

Figure 3. Temperature dependence of the *D* values for the thermal degradation of L-ascorbic acid in squeezed tomatoes, variety A (∗) and B (△), and in squeezed oranges (#).

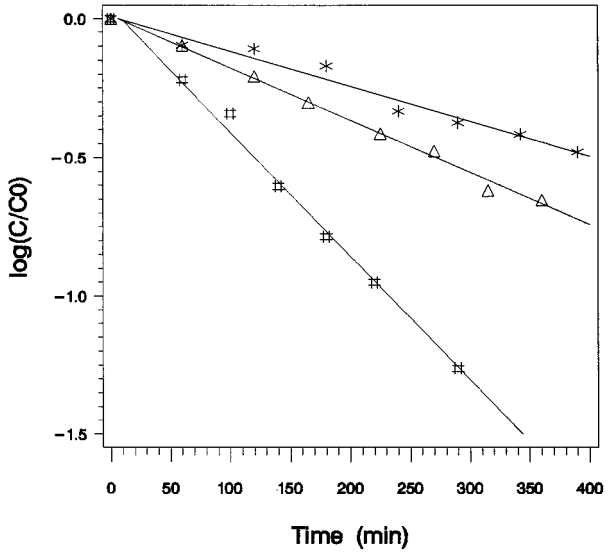

Figure 4. Pressure−temperature degradation kinetics of L-ascorbic acid in squeezed oranges at 8500 bar and 65 (∗), 70 (△), and 80 (#) °C.

Table 3. *D*, *k*, *z*, and E_a Values for the Thermal Degradation of L-Ascorbic Acid (2 mg/mL) in Squeezed Oranges

	oranges		
T (°C)	D (min)	$k \times 10^{-1}$ (min^{-1})	r^2
120	302.94 ± 12.65[a]	0.076 ± 0.003	0.99
130	112.38 ± 7.12	0.205 ± 0.014	0.98
140	47.93 ± 2.48	0.480 ± 0.026	0.98
150	23.81 ± 1.07	0.967 ± 0.046	0.99

z (°C)	E_a (kcal/mol)	r^2
27.15 ± 1.40	28.1 ± 1.1	0.99

[a] Standard error.

that variations in ascorbic acid retention were dependent upon individual crop. Based on a 95% confidence interval, the difference between the *z* value of L-ascorbic acid degradation in oranges and tomatoes (varieties A and B) is not significant. For both oranges and tomatoes, a temperature increase of approximately 30 °C is needed to decrease the *D* value by 1 log unit. This value is comparable to the *z* value for other vitamins in a similar temperature range (Villota and Hawkes, 1986).

To be able to compare the kinetic parameters, estimated in this study, with earlier reported values describing thermal processing (and not the effect of temperature during storage), the more classical *k* and E_a values are also given in Tables 2 and 3. Lathrop and Leung (1980) reported a k_{110} value of 0.5×10^{-3} min^{-1} for the overall degradation of ascorbic acid (aerobic and anaerobic) during thermal treatment of peas and an activation energy E_a, determined in a temperature range from 110 to 132 °C, of 41 kcal/mol. From these values, k_{121} can be estimated as 0.2×10^{-2} min^{-1}. Rao et al. (1981) reported a k_{121} of 0.9×10^{-2} min^{-1} (D_{121} = 246 min) for the degradation of ascorbic acid during thermal treatment of peas and an activation energy of 13.1 kcal/mol. Laing et al. (1978) studied the degradation kinetics of ascorbic acid in different model food systems in the temperature range 61−105 °C. They found an activation energy, E_a, in the range 14−17 kcal/mol. On the basis of the differences between earlier reported values and the values we estimated, we assume that the mechanism for ascorbic acid degradation in the products was different. Factors that could contribute to these differences include oxygen levels and chemical aspects (such as pH) as a result of type of product (variety and degree of maturity).

Isobaric−Isothermal Degradation Kinetics of L-Ascorbic Acid in Squeezed Oranges and Tomatoes. A combined pressure−temperature treatment was applied to determine the antagonistic or synergistic effect of pressure and temperature on the degradation of L-ascorbic acid in squeezed oranges (2 mg/mL) and tomatoes (2.4 mg/mL). At a pressure of 8500 bar, samples of L-ascorbic acid were subjected to temperatures ranging from 65 to 80 °C. In all cases, the pressure−temperature degradation of L-ascorbic acid could be accurately described by a first-order reaction, as can be seen from the straight lines of $\log(C/C_0)$ versus time (see Figure 4 for the degradation of L-ascorbic acid in squeezed oranges). The temperature dependence of

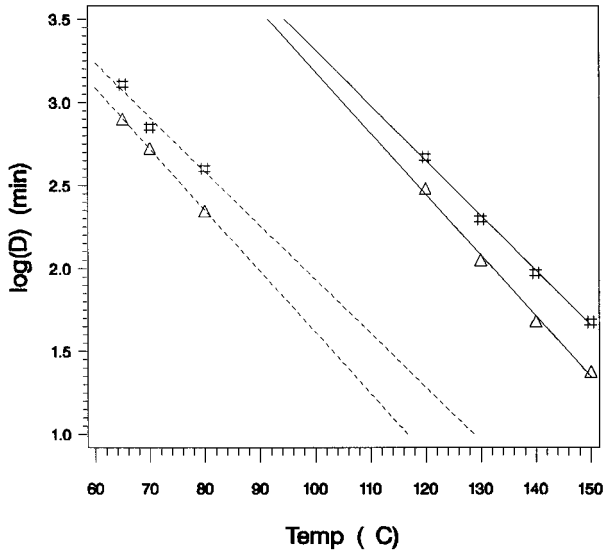

Figure 5. Temperature dependence of the *D* value for the thermal degradation of L-ascorbic acid in squeezed oranges at 1 bar (−△−) and 8500 bar (- -△- -) and in squeezed tomatoes at 1 bar (−#−) and 8500 bar (- -#- -).

Table 4. *D* and *z* Values for the Combined Pressure−Temperature Degradation of L-Ascorbic Acid in Squeezed Tomatoes and Oranges (Applied Pressure, 8500 bar)

	D (min)	
T (°C)	tomatoes, variety A	oranges
65	1287.0 ± 106.2[a]	794.9 ± 60.6[a]
70	711.7 ± 35.9	530.8 ± 16.2
80	400.9 ± 27.6	223.8 ± 7.9
	z (°C)	
	30.77 ± 5.17	27.16 ± 0.45

[a] Standard error.

the *D* value is given by the *z* value, which is illustrated in Figure 5. The estimated kinetic parameters are given in Table 4.

From Table 4, it is clear that pressure and temperature act synergistically on the degradation of L-ascorbic acid in the pressure−temperature domain studied; the rate of L-ascorbic acid degradation at 8500 bar increases with increasing temperature from 65 to 80 °C. Similarly to thermal treatment, the sensitivity of L-ascorbic acid toward pressure and temperature depend on the environment. From Table 4 it can be deduced that L-ascorbic acid is more sensitive in oranges than in tomatoes, which is in agreement with results obtained from thermal experiments. The differences in *z* value at elevated pressure between oranges and tomatoes are not so pronounced, and they are very similar to the *z* value at atmospheric pressure; in other words, the temperature sensitivity of the *D* value is not dependent on the pressure level. This is graphically illustrated in Figure 5. From this figure it can also be deduced that the temperature domain in which L-ascorbic acid degrades shifts to lower values (about 40 °C lower) as a pressure of 8500 bar is applied.

An attempt was made to determine the concentration of the dissolved oxygen in the juice in order to find out whether ascorbic acid degraded aerobically or anaerobically. Measurement of dissolved oxygen concentration, before and after different pressure−temperature−time treatments, revealed that there was no consumption of oxygen during the pressure−temperature treatment, from which we conclude that L-ascorbic acid degraded anaerobically. This assumption was confirmed by comparing the *D* values of L-ascorbic acid degraded in juice without prior treatment on the one hand and subjected to nitrogen flush on the other hand. Based on a 95% confidence interval, neither estimated *D* value differed significantly from the other.

From the pressure−temperature experiments performed, it can be derived that L-ascorbic acid is unstable at a combination of relatively high pressure (8500 bar) and temperature (60−85 °C). At the same pressure level and lower temperature (50 °C), no degradation of L-ascorbic acid was observed within 1 h. We can conclude that at mild treatments, which are of importance in the industry at this moment, L-ascorbic acid will be kept. Similar conclusions can be found in the literature. Donsi et al. (1996) performed pressure experiments on orange juice to stabilize this product. He found that the product was fully stabilized for commercial purposes at a pressure not lower than 3500 bar during 1 min and reported that high-pressure treatment had no significant effect on ascorbic acid content of the juice. Also Takahashi et al. (1993) and Ogawa et al. (1992) found no change in ascorbic acid concentration after pressurization of citrus juice up to 6 kbar at room temperature. Quaglia et al. (1996) studied the effect of high pressure (4000−9000 bar) on green peas. They found that an increase in pressure resulted in a higher level of ascorbic acid retention. About 82% of the original ascorbic acid content remained in green peas after treating at 9000 bar.

ABBREVIATIONS USED

MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; PMS, 5-methylphenazizium methyl sulfate; AAO, ascorbic acid oxidase.

SYMBOLS

C = concentration (mg/mL)
C_0 = initial concentration (mg/mL)
D = decimal reduction time (min)
E_a = activation energy (kcal/mol)
k = inactivation rate constant (1/min)
k_{ref} = inactivation rate constant at a reference temperature (1/min)
P = pressure (bar)
R = universal gas constant (= 1.9872 cal/mol K)
t = time (min)
T = temperature (K)
T_{ref} = reference temperature (K)
z = z value (°C)

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