

Kinetic and prediction studies of ascorbic acid degradation in normal and concentrate local lemon juice during storage

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Abstract

Kinetics of the loss of ascorbic acid (AA) in local lemon juice of 9° and 50° Brix stored at 25, 35, and 45 °C for 4 months have been investigated. The results indicate that increases of concentrations and temperatures increase the rate of AA degradation. The calculated values of activation energies (E^{\ddagger}) and frequency factors (A) at different Brix imply that the concentration of juice does not change the mechanism of degradation. Thermodynamic functions of activation (ΔG^{\ddagger} , ΔH^{\ddagger} , ΔS^{\ddagger} and K^{\ddagger}) have been determined and considered briefly. A direct equation for estimation of the shelf life of stored juice with respect to first-order losses of AA, at any specific temperature and degradation ratio, has been derived and programmed successfully.

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1. Introduction

It is well known that ascorbic acid (AA) or vitamin C is present in most natural juices and is used extensively in the food industry. The latter is not only for nutritional value but also for many functional contributions of AA to product quality. On the other hand, AA degradation in foods is a widespread phenomenon that take place during processing and storage (Manso, Oliveria, Oliveria, & Frias, 2001). Such degradation affects the sensory characteristics of processed food, such as flavour and colour (Rassis & Saguy, 1995). However, AA degradation reactions are often responsible for important quality changes that occur during the storage of foods, limiting their shelf-life (Rojas & Gerschenson, 1997), with formation of unstable intermediate compounds, such as furfural (Robertson & Saminego-Esguerra, 1990).

Indeed, the degradation of AA was reported to be the major deteriorative reaction that occurs during the storage of citrus juices (Lee & Nagy, 1988). Such a phenomenon has been investigated by a number of researchers (Manso et al., 2001; Rassis & Saguy, 1995; Robertson & Saminego-Esguerra, 1986, 1990; Ziena, 2000). It has been assumed that the kinetics of AA degradation are highly variable and depend on several factors. In other words, zero-, first-, and second-order rate constants of AA degradation have been reported (Koca, Burdurlu, & Karadeniz, 2003; Liao & Seib, 1988; Robertson & Saminego-Esguerra, 1986, 1990; Rojas & Gerschenson, 1997; Singh, Heldman, & Kirk, 1976). This gave a reasonable theoretical explanation for the order of the degradation reaction.

The main objective of the present work is to determine the kinetics of AA degradation by determining the thermodynamic functions of single strength (9° brix) and concentrate (50° brix) of local lemon juice during storage at 25, 35, and 45 °C for 4 months. These thermodynamic functions might give helpful information about the degradation phenomenon. The work also reports a direct equation, derived

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Nomenclature

A	frequency factor	k_B	Boltzmann constant
AA	ascorbic acid	\ln	natural logarithm
$[A]_0$	initial concentration of AA	$P\%$	percent of AA residual
$[A]_t$	concentration of AA at time t	Q_{10}	$Q_{10} = \left[\frac{k_{at2}}{k_{at1}} \right]^{\frac{10}{T_2-T_1}}$
ΔG^\ddagger	free energy of activation	R	thermodynamic gas constant
ΔH^\ddagger	enthalpy of activation	r	correlation coefficient
ΔS^\ddagger	entropy of activation	r^2	square correlation coefficient
E^\ddagger	activation energy	S	slope of Arrhenius equation
e	mathematical exponential	T	absolute or Kelvin temperature
Ex	shelf life of the product in time unit	t	time
h	Plank's constant	TSS	total soluble solids
I	intercept of Arrhenius equation	v	constant = $k_B T/h$
k	first-order rate constant		
K^\ddagger	equilibrium of activation (between reactant and activated complex)		

for estimation of the shelf life of stored food with respect to AA at any specific temperature and degradation ratio.

2. Materials and methods

Lemon fruits (citrus limon var local) were divided into two halves, the juice was extracted using a Sanyo-electrical rotary extractor, and then rapidly strained through a cheese-cloth and stainless steel sieve with pore diameter of 125 μm to accomplish separation of most of the suspended matter from juice. The latter was divided into two portions; the first one was pasteurized at 65 °C for 30 s (Nagy, Shaw, & Veldhui, 1977), while the second portion was concentrated, using a rotary vacuum evaporator until it reached about 50° brix. The two types of juices were stored in glass bottles (closed by rubber caps) at 25, 35, and 45 °C for 120 days. The rubber caps were tightly surrounded by aluminium foil which may prevent oxygen permeation. However, dipping rubber caps in paraffin wax is more favourable.

Total soluble solids (TSS) were determined using an Abbe refractometer at 20 °C (Rangana, 1977). Total titrable acidity was assessed by titration with sodium hydroxide (0.1 N) and expressed as % citric acid (Rangana, 1977).

AA was determined using 2,6-dichlorophenolindophenol by visual titration (AOAC, 1980); these properties were measured monthly with three replications. Data of the determined properties were subjected to analysis of variance and Duncan's multiple range test using the SAS system (1982). Zero-, first-, and second-order integrated rate equations (Houston, 2001) were applied to the results of AA concentration versus time and the appropriate method was selected on the basis of the value of the squared correlation coefficient (r^2) obtained from regression analysis using Microsoft Excel 2002 software.

2.1. Calculations

A direct equation for estimation the shelf life of stored lemon juice or any food with respect to the loss in AA content at any specific temperature and degradation ratio has been derived and presented by following equation:

$$\text{Ex} = e^{-\left[\frac{S}{T} + I - \ln\left(-\ln\left(\frac{p\%}{100}\right)\right)\right]} \quad (1)$$

where Ex represents the shelf life of the product (in hours, days, weeks), depending on the unit of the first-order rate constant; S and I are the slope and intercept obtained from the application of the Arrhenius equation through the plot between logarithm of first-order rate constant versus $1/T$; T is the absolute or Kelvin temperature and $p\%$ is the percent of residual AA or the residual of any determined material. For example, one could predict the period of time needed to consume 10% of AA. Therefore, the residual is 90% ($p\% = 90$) and the derived equation becomes as follows with respect to the data listed in Table 2 for 9° Brix at 25 °C.

$$\text{Ex} = e^{-\left[\frac{-6936.185}{298.15} + 19.7066 - \ln\left(-\ln\left(\frac{90}{100}\right)\right)\right]} = 3.695 \text{ months} \quad (2)$$

The above presented Eq. (1) was derived as follows:

From the integral equation of first-order reaction (Houston, 2001),

$$kt = \ln \frac{[A]_0}{[A]_t} \quad (3)$$

where k , t , $[A]_0$, and $[A]_t$ are first-order rate constant, time, initial concentration of AA, and its concentration at time t (the residual of AA at time t), respectively. Eq. (3) can be represented as

$$kt = \ln[A]_0 - \ln[A]_t \quad (4)$$

Dividing both $[A]_0$ and $[A]_t$ of the right side of Eq. (3) by 100 does not perturb its value. Let the initial concentration

$[A]_0$ be equal to 100; therefore, $\ln[A]_0$ in Eq. (4) will be equal to zero and the last Eq. (4) can be written as

$$kt = -\ln \frac{[A]_t}{100} \quad (5)$$

Let $p\%$ be the residual percent of $[A]_t$; therefore Eq. (5) can be represented as:

$$k = \frac{-\ln(p\%/100)}{t} \quad (6)$$

The natural logarithm of Eq. (6) can be written as

$$\ln k = \ln(-\ln(p\%/100)) - \ln t \quad (7)$$

On the other hand, the Arrhenius equation is expressed as (Houston, 2001),

$$\ln k = \ln A - \frac{E^\ddagger}{R} \quad (8)$$

where A , E^\ddagger , and R are frequency factor, activation energy and gas constant, respectively. Let I be $(\ln A)$ and let S be $(-\frac{E^\ddagger}{R})$ which both represent the intercept and slope of the plot between $\ln k$ against $1/T$ of Eq. (8), respectively. Thus, Eq. (8) is written as

$$\ln k = I + \frac{S}{T} \quad (9)$$

The left sides of Eqs. (7) and (9) are equal; therefore, combination of those equations yields

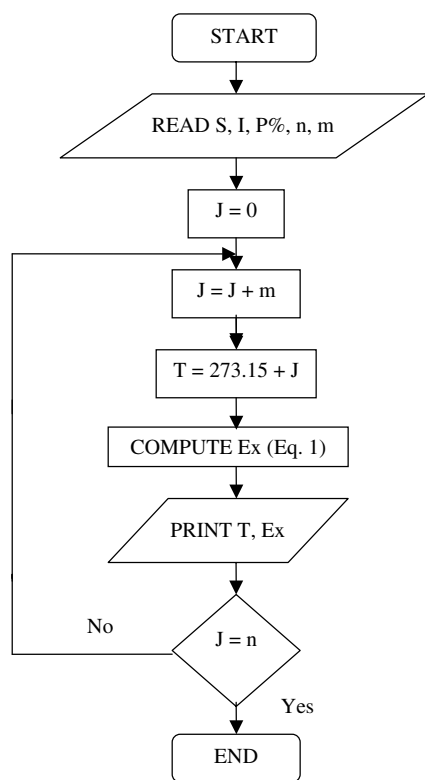


Fig. 1. Flowchart of the computer programme for determining AA shelf life according to Eq. (1), where n and m present the maximum value of temperature and its interval, respectively.

$$\ln(-\ln(p\%/100)) - \ln t = I + \frac{S}{T} \quad (10)$$

Let Ex be the shelf life (t); then, rearrangement of Eq. (10) can lead to the above derived Eq. (1).

A computer programme for the application of the derived Eq. (1) has been written using Microsoft Visual Basic 6 software with the flowchart exhibited in Fig. 1.

3. Results and discussion

Zero-, first- and second-order kinetic models were used to treat the resulting data of AA degradation during storage as represented in Fig. 2. Since regression analysis of logarithm AA retention and time gave a higher linear relationship, a first-order kinetic model was used to determine AA data in our study. In fact, the first-order kinetic model has been applied by Kanner and coworkers (1982) for evaluating AA degradation in 58° brix orange juice during storage at 25–35 °C. Manso et al. (2001) reported that the degradation of AA in 11° brix orange juice also followed first-order reaction kinetics at 20, 25, 30, 35, 40, and 45 °C. From the fundamental reaction kinetics point of view, the first-order model is more reasonable than the zero- and second-order models. This could be because, if the degradation is following zero-order with respect to AA concentration, its rate must not depend on juice concentration and should give similar values of rate constants. On the other hand, when the reaction is second-order with respect to AA, this means that the more probable order of the whole reaction is third-order, as certainly there is another factor involved. But, the latter order of reaction is quite rare and very slow under natural conditions as there must be three molecules colliding simultaneously (Avery, 1974).

The results of computation by the first-order reaction kinetic model are listed in Table 1. These show that there

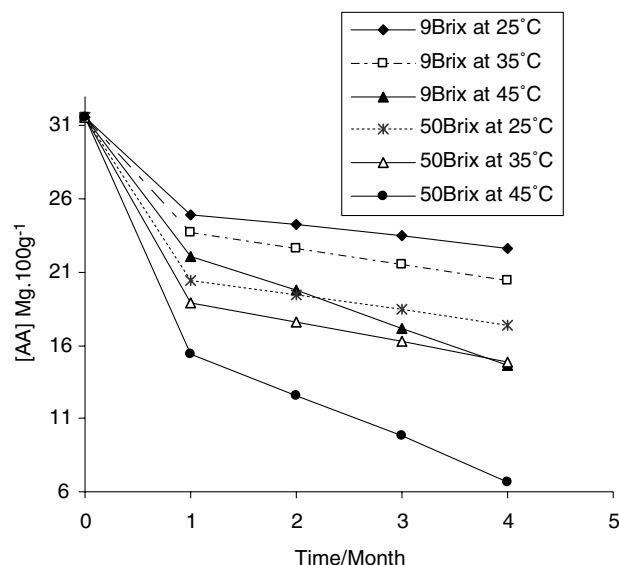


Fig. 2. Change of AA concentration with time during storage at different concentrations and temperatures.

Table 1
Kinetic parameters for two concentrations of stored local lemon juice

Sample concentration	Temperature (°C)	k (month ⁻¹)	r^2	Q_{10}			Rate (mg l ⁻¹ month ⁻¹)
				25–35	25–45	35–45	
9° brix	25	0.0315	0.995	1.568	2.084	2.771	9.95
	35	0.0494	0.999				15.6
	45	0.137	0.995				43.3
50° brix	25	0.0535	0.998	1.489	2.264	3.443	16.9
	35	0.0797	0.997				25.1
	45	0.274	0.980				86.7

Total soluble solids of fresh lemon juice = 9.20%, did not change significantly during storage at 25, 35 and 45 °C. Total titrable acidity of fresh lemon juice = 5.5% (as citric acid), did not change significantly during storage at 25, 35 and 45 °C. Ascorbic acid of fresh lemon juice = 31.61 mg/100 ml, did change significantly for single-strength and concentrated during storage at 25, 35 and 45 °C.

is a remarkable increase in the rate of degradation caused by increase of both temperature and juice concentration. Such a phenomenon is merely consistent with the fundamentals of chemical kinetics and arises mainly from the increases in the number of collisions caused by concentration and temperature. Saguy, Kopelman, and Mizrahi (1978) observed that, at constant temperature, the rate of AA degradation in grapefruit increased steadily with TSS. Similar results were also obtained by Kanner, Fishbein, Shalom, Harel, and Ben-Gare (1982) during the storage of orange juice. On the other hand, the results of Robertson and Saminego-Esquerria (1990) somewhat disagree with our present results. The reason for this may be their relatively low correlation coefficients (r^2) ranging between 0.032 and 0.962. However, those workers (Robertson & Saminego-Esquerria, 1990) found that, at 36 °C, the 50° brix sample had a higher rate constant than had those at 40, 30, and 20° brix lemon juice.

The values of Q_{10} (Table 1) indicate that there is a substantial increase in rate constant when the temperature exceeds 35 °C. The relatively low value of $Q_{10}(25-35)$ of 50° brix in contrast to that of 9° brix suggests the significance of molecular associations which could decrease the rate of AA degradation at temperatures below 35 °C. The latter effect can be confirmed by determining the activation energies and other related thermodynamic functions of this degradation.

Table 2 lists the results of application of the Arrhenius Eq. (8) to the above kinetic results. The 50° brix sample has a higher value of E^\ddagger which confirms the significance of molecular associations. The values of the frequency factor indicate that an increase in juice concentration from 9 to 50° Brix magnifies its value by 22-fold which can give the proportionality between rate constant and juice concentration. Thus, the rate constant depends substantially

Table 2
Results of application of Arrhenius equation to kinetic data

Sample	Slope	Intercept	r (Corr. Coef.)	E^\ddagger (kcal mol ⁻¹)	A (month ⁻¹)
9° brix	-6936	19.7066	0.971	13.8	3.62×10^8
50° brix	-7711	22.7888	0.954	15.3	7.89×10^9

upon the number of collisions (A). The latter could provide an excess of energy that can deliver the molecules to the activation state and then increase the rate of degradation (Avery, 1974; Houston, 2001).

Thermodynamic functions of activation have been measured at 25 °C and are illustrated in Table 3. These show an extreme physical significance, which can be helpful for realizing some aspects of AA degradation. The close values of ΔG^\ddagger indicate a similar factors affecting the degradation rate in both dilute and concentrated of juice (similar degradation mechanism) in which the value of ΔG^\ddagger represents the difference between the activated state and reactants, and therefore must have a positive sign. The sign of ΔH^\ddagger represents an endothermic state between activated complex and reactant which leads to increase of the degradation with increasing temperature. The lower value of ΔH^\ddagger for 50° brix, in contrast to that for 9° brix suggests that the above mentioned molecular associations occur mainly in the concentrated sample between solute molecules. For 9° brix, this indicates that the molecular association is formed between solvent and solute molecules (Laidler, 1980). Thus, It could be suggested that the solute molecules, such as AA, fructose and citric acid, in concentrated juice, associate with each other through inter-molecular hydrogen bonds to make a structure like a cage, which is responsible for the values of $Q_{10}(25-35)$, E^\ddagger , and ΔH^\ddagger (Tables 1–3). Such a cage effect could reduce the degradation at low temperature but it will decompose over 30 °C. The relatively low value of ΔS^\ddagger implies the law significance of this function, while its negative sign indicates an increase in order as a

Table 3
Thermodynamic functions of activation for the degradation of AA in local lemon juice at 25 °C

Sample	ΔG^\ddagger ^a (kcal mol ⁻¹)	ΔH^\ddagger ^b (kcal mol ⁻¹)	ΔS^\ddagger ^c (cal mol ⁻¹ deg ⁻¹)	K^\ddagger ^d
9° brix	19.5	13.1962	-21.2	5.073×10^{-15}
50° brix	19.2	14.736	-15.0	8.62×10^{-15}

^a $k(\text{month}^{-1}) = v \exp(-\Delta G^\ddagger/RT)$.

^b $\Delta H^\ddagger = E^\ddagger - nRT$; ($n = 1$).

^c $\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger$.

^d $k(\text{month}^{-1}) = v K_{\text{eq}}^\ddagger$, $v(\text{constant}) = k_B T/h$.

result of forming an activated complex. The lower value of ΔS^\ddagger at 9° brix in contrast to 50° brix may be attributed to a relatively less ordered state in high concentration juice, due to an increase in the number of molecules. The values of K^\ddagger indicate that there is about one molecule of AA activated and degraded for 10^{15} inactivated molecules.

It should be noted that the enthalpy (ΔH^\ddagger) and entropy (ΔS^\ddagger) of activations have been reported previously by Labuza (1980). In fact, the results of such $\Delta H^\ddagger - \Delta S^\ddagger$ compensation are quite statistical and appear to be independent of the physical and chemical patterns of studied reactions and equilibria (Kurg, Hunter, & Grieger, 1976).

Table 4 lists the results of application of the derived Eq. (1) for prediction of the shelf life or the period of time required for 25% and 50% loss of AA ($p\% = 75$ and 50, respectively) for 9° and 50° brix at temperatures ranging from 0 to 60 °C with intervals of 5 °C. Thus, one could predict the shelf life when one knows exactly (from any authorized committee) the permitted minimum loss ratio of AA using Eq. (1). Application of Eq. (1) has been extended

Table 4
Calculated shelf life of 9° and 50° brix local lemon juice at different temperatures

Temperature (°C)	9° brix		50° brix	
	$p\% = 75$	$p\% = 50$	$p\% = 75$	$p\% = 50$
	25% Loss of vitamin C (month)	50% Loss of vitamin C (month)	25% Loss of vitamin C (month)	50% Loss of vitamin C (month)
0	84.8	204	66.2	160
5	53.7	130	39.9	96.1
10	34.6	83.3	24.4	58.9
15	22.6	54.4	15.2	36.7
20	15.0	36.1	9.66	23.2
25	10.0	24.3	6.21	14.9
30	6.87	16.5	4.05	9.77
35	4.74	11.4	2.68	6.46
40	3.31	7.97	1.80	4.33
45	2.33	5.63	1.22	2.94
50	1.66	4.01	0.84	2.02
55	1.20	2.89	0.58	1.40
60	0.87	2.11	0.41	0.99

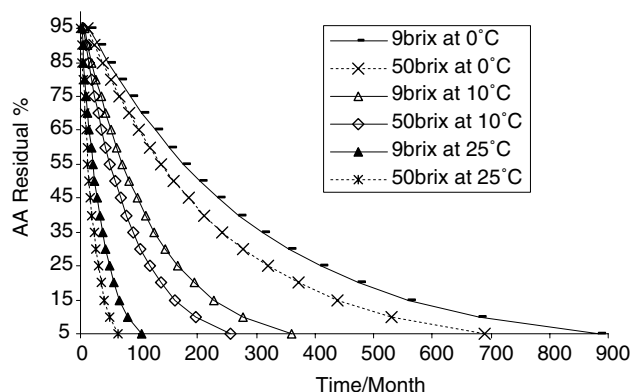


Fig. 3. Plot of residual% AA versus time in month unit for 9° and 50° Brix at 0, 10, and 25 °C resulting by application of the derived Eq. (1).

by calculation of the time needed for residual% AA at 0, 10, and 25 °C for both 9° and 50° brix. The results of this application are illustrated clearly in Fig. 3. These indicate that the effect of temperature upon the rate of degradation is more significant than that of concentration. Such indication has also been realized by Eskin (1990).

4. Conclusions

On the basis of the results we can conclude the following points:

1. The degradation process is dominated by a collision factor which increases by increasing both concentration and temperature.
2. The total soluble solids (TSS) have no catalytical effect on AA degradation (the catalyst must reduce the activation energy of chemical reactions).
3. It has been suggested that at relatively low temperature, there is a cage effect produced by molecular associations of solutes at high concentration which can somewhat reduce the degradation process.
4. Thermodynamic functions can be measured for food reactions and may give valuable information.
5. A useful equation for prediction of the shelf life, with respect to a first-order kinetics model, has been derived. If one found, in other food reactions, that the process does not follow first-order, one could derive another equation in a similar manner for zero- or second-order processes.

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