Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuaçu (*Theobroma grandiflorum*) nectar

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Abstract

The thermal degradation kinetics of both components of vitamin C, ascorbic acid (AA) and dehydroascorbic acid (DHAA), were determined in a nectar of Cupuaçu (*Theobroma grandiflorum*) with 25% of pulp and 15% of sugar in water. AA was assayed by HPLC and the results showed that AA degraded into DHAA. A reversible first order model described well the AA degradation data, with an activation energy of 74 ± 5 kJ/mol and $k_{80^\circ C} = 0.032 \pm 0.003\,\text{min}^{-1}$. DHAA kinetic behavior suggested a consecutive first order reaction where DHAA was the intermediate product of AA degradation. A mechanistic model was derived to predict DHAA concentration. Rate constants were replaced by the Arrhenius equation in the model to evaluate the temperature dependence and the kinetic parameters for AA degradation, previously determined, were used. An activation energy of 65 ± 9 kJ/mol and a $k_{80^\circ C}$ of 0.013 ± 0.003 min^{-1} were estimated. The present findings will help to predict the best Cupuaçu nectar processing conditions that minimize degradation of an important quality factor such as vitamin C.

Keywords: Cupuaçu; Ascorbic acid; Dehydroascorbic acid; Thermal treatment kinetics; Modeling

Nomenclature

AA	ascorbic acid
C	concentration
D	decimal reduction time (min)
DHAA	dehydroascorbic acid
DKGA	deketogulonic acid
Ea	activation energy (kJ/mol)
k	rate of the reaction (min^{-1})
R	Universal gas constant ($=8.314$ kJ/mol K)
T	absolute temperature (K)
t	time (min)
z	z-value (°C)

Subscripts

1	relative to ascorbic acid
2	relative to dehydroascorbic acid
80°C	at the reference temperature of 80°C
0	initial value at time equal to zero
AA	ascorbic acid
DHAA	dehydroascorbic acid
DKGA	deketogulonic acid
∞	final value at equilibrium
ref	at the reference temperature

Superscripts

*	value relative to the equilibrium

1. Introduction

Cupuaçu is an exotic Brazilian fruit of the genera *Theobroma* (*Theobroma grandiflorum Shum*) from the family of cacao (*Theobroma cacao*) that grows spontaneously in the South and Southwest of Pará as well as in the pre-amazonian area of Maranhão (Venturieri, 1993). Cupuaçu is a very acid fruit (pH $\cong$ 3.2), low in sugar (Chaar, 1980) and has a very strong aroma. Cupuaçu pulp is used to flavor food products such as jellies (Santos et al., 1995), yogurts (Pina & Ribeiro, 1995; Santos et al., 1995) and candies (Lopes & Neves, 1995). By adding water and sugar a very pleasant exotic nectar is obtained. Due to its low pH value a mild thermal treatment, such as a pasteurization process, should stabilize this product at room temperature (Lund, 1976). However, a thermal treatment always degrades the product original quality to a certain extent. The more important nutritious attribute so far detected in Cupuaçu is vitamin C, 15–28 mg of ascorbic acid/100 g of pulp (Barbosa, Nazaré & Soares, 1978; Chaar, 1980; Miranda, 1989).

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Ascorbic acid is known to be thermolabile. To date several authors have studied its thermal degradation kinetics in citric juices under pasteurization conditions and stated that it follows a first order reaction model (Table 1) (Johnson, Braddock & Chen 1995; Saguy, Kopelman & Mizrah, 1978). Alvarado and Viteri (1989) studied the degradation of total vitamin C in citric fruits for an extended time range (up to 150 min at a temperature of 60°C) and concluded also that the reaction was first order, (Table 1). No studies were published so far on the pattern that ascorbic acid (AA) degradation follows when the time range is extended far beyond the practical range under pasteurization conditions.

The vitamin C degradation mechanism is specific of a particular system, as it depends on several factors (Tannenbaum, 1976), following either an aerobic or anaerobic pathway. In the aerobic pathway oxidation can follow a catalyzed pathway due to the presence of metals, or an uncatalyzed pathway. Both pathways have common intermediate products that cannot be distinguished by chemical analysis and both lead to dehydroascorbic acid (DHAA) which by further degradation forms 2,3-diketogulonic acid (DKGA). In the anaerobic pathway ascorbic acid undergoes ketonization to form the intermediate keto-tautomer (keto-ascorbic acid) which is in equilibrium with its anion (keto-monoanion ascorbic acid) which by further delactonization forms DKGA (Tannenbaum, 1976).

Work on the influence of pH, oxygen, (Eison-Perchonok & Downes, 1982), cupric-ion (Hsieh & Harris, 1987; Sahbaz & Somer, 1993), sucrose (Hsieh & Harris, 1987; Hsieh & Harris, 1993), enzymes and aminoacids (Jung, Kim & Kim, 1995) on the rate of AA degradation greatly contributed to a better knowledge of the behavior of ascorbic acid in different systems during storage.

In fruit juices processing, AA can suppress enzymatic browning by reversing the oxidation of polyphenols to o-quinones that through polymerization form brown pigments (Sawamura, Takemoto, Matsuzaki, Ukeda & Kusunose, 1994). DHAA, the other biologically active form of vitamin C, also found in many fruits and vegetables in nature, is readily obtained by oxidation of AA, a reaction that depends on heat and oxygen (Bradbury & Singh, 1986). In citrus products the browning process is not enzymatic, starting with the degradation of DHAA to form several ozones which through the Maillard reaction produce browning compounds regardless of the presence of oxygen. The occurrence of browning can therefore be avoided by preventing DHAA from degrading (Sawamura et al., 1994).

Inspite of the importance of the antioxidant properties of DHAA, most published data on fruits and vegetables refer only to AA as vitamin C. This can be explained once we find that, until recently, the most commonly used analytical methods to determine vitamin C used a redox titration (Dichlorophenolindophenol method) or a direct treatment with phenylhydrazine to form a derivative detectable by spectrophotometry, and AA is the only form assayed this way. To date limited information is available on the kinetics of DHAA degradation in foods under thermal treatment.

In order to be able to evaluate the process impact on Cupuaçu nectar quality, the main objective of this study was the mathematical modeling of the thermal degradation of vitamin C in both forms (ascorbic and dehydroascorbic acids) in Cupuaçu nectar using an isothermal method.

2. Materials and methods

2.1. Thermal treatment in TDT tubes

Cupuaçu pulp was imported frozen from Belém, Brazil, and stored at −20°C. Just before the experiments it was taken out of the freezer and cut into small chunks. Refined sugar plus deionized water were then added to the pulp in order to obtain a nectar with the formulation appreciated in the State of Pará, in Brazil (25% pulp and 15% sugar). Preliminary sensory experiments confirmed

Table 1
Kinetic parameters of thermal degradation of ascorbic acid in other fruits

Product	Temperature range	pH	°Brix	*Ea* (kJ/mol)	k_0[a] (s^{-1})	*z* (°C)	$D_{75°C}$ (min)	Reference
Grapefruit juice	61.0–96.0	3.05	11.2	21.0	3.90×10^{-2}	49.3	1354	Saguy et al. (1978)
Grapefruit juice	60.0–91.0	3.05	31.2	22.0	6.10×10^{-2}	45	1228	Saguy et al. (1978)
Lime	20.0–92.0	5.92	6.3	58.1	1.55×10^{4}	35.8	1186	Alvarado and Viteri (1989)
Lemon (Oriente)	20.0–92.0	2.94	6.0	46.5	3.59×10^{2}	44.6	949	Alvarado and Viteri (1989)
Tangerine (Costa)	20.0–92.0	4.10	13.4	44.6	2.25×10^{2}	46.5	771	Alvarado and Viteri (1989)
Grapefruit	20.0–92.0	3.54	11.2	56.9	9.29×10^{3}	36.5	1276	Alvarado and Viteri (1989)
Orange juice	70.3–97.6	3.60	12.5	128.3	3.23×10^{13}	19.0	24110	Johnson et al. (1995)
Orange juice	70.3–97.6	3.60	36.7	97.4	1.62×10^{9}	24.9	10447	Johnson et al. (1995)

[a] Rate constant at infinite reference temperature.

that this is the optimum mixture in terms of consumer acceptance. The mixture was homogenized with a Moulinex Turbomix 2 blender during 5 min and passed through a plastic screen. Brix and pH were measured with an Atago hand refractometer and a Crison micropH meter 2001, respectively. Five ml of nectar were pipetted into thermal death time (TDT) tubes (95 mm length, 10 mm i.d.) leaving a headspace of 2 cm. Two replicates were used per experiment. The tubes were stoppered, placed alternated in a rack and immersed in a Thermomix (±1°C) water bath previously set at the desired temperature. The tubes were taken out of the water bath (two at a time, randomly chosen) after the required holding time and immediately cooled down to 4°C in an iced water bath to stop the heat treatment immediately. Six temperatures were studied (60°C, 70°C, 75°C, 80°C, 90°C and 99°C) for holding times ranging from 0 to 240 min.

2.2. Simultaneous analysis of ascorbic and dehydroascorbic acid by reverse phase ion interaction HPLC

Ascorbic acid (Merck) and dehydroascorbic acid (Aldrich) content were determined experimentally by HPLC UV detection and using isoascorbic acid (IAA) (Fluka) as internal standard (Zapata & Dufour, 1992). DHAA was detected as fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxaline-1-one (DFQ) which absorbs in the UV region (348 nm) after pre-column derivatization with 1,2-phenylenediamine dihydrochloride (OPDA) (Aldrich). After heat treatment each sample was transferred to a 20 ml volumetric flask, the TDT tube was washed twice with a methanol (Merck)/water (ultrapure) solution (5:95). One ml of isoascorbic acid internal standard solution (0.03 mg/50 ml) was added together with 100 μl of 1 M HCl (Merck) solution in order to bring the pH down to 2.5. The volume was then completed to 20 ml with the methanol/water solution. Part of the flask content was then transferred to a centrifuge (Spectra Merlin) tube and centrifuged for 5 min at 1610 g. Following this, 3 ml were pipetted to another tube together with 1 ml of 1,2-phenylenediamine dihydrochloride (OPDA) (Aldrich) solution (0.03 g/50 ml) daily prepared and always kept in the dark. Next, the tube was vortexed and placed in the dark at environmental temperature. After 37 min the tube was taken out and the contents filtered through a 0.22 μm filter (Milipore) to a 2 ml screw cap vial (Chrompack). The first milliliter was discarded. Ascorbic and dehydroascorbic acids were then determined by HPLC. The detector wavelength was first set to 348 nm until 5 min for detection of the derivatized DHAA and then automatically shifted to 262 nm for AA and IAA detection. The HPLC system consisted of a Beckman System Gold™ HPLC (Beckman Instruments, Fullerton, CA, USA) composed of a pump (Programmable Solvent Module 126), a 20 μl injector loop, a pre-column (KS 30/4 Nucleosil 120-5 C18) followed by a C18 column (Spherisorb 50DS2, 25 cm × 4.6 mm i.d.) and a UV detector (Programmable Detector Module166).

As Cupuaçu AA and DHAA initial concentrations vary slightly from batch to batch of nectar, it was decided that ascorbic acid and dehydroascorbic acid degradation should always be evaluated in a relative way. Therefore, in each experiment the initial concentration of ascorbic acid and dehydroascorbic acid in the raw nectar were determined and the observed concentrations after the thermal treatment were divided by the initial concentrations in order to obtain relative degradations.

2.3. Degradation kinetics modeling

Rate constants were determined by non-linear regression of concentration as a function of time at constant temperature. The kinetic parameters were then estimated based on the Arrhenius law (two-step analysis) and the adequacy of the models studied. A one-step procedure was then tried to reduce standard deviation in *Ea* (Arabshahi & Lund, 1985), and a non-linear regression was performed through all the data points in order to calculate *Ea* from the original data. In all this study the Statistical Software STATA version 4.0 (Statacorp, 1995) was used.

3. Results and discussion

3.1. Vitamin C content

25–35 mg of ascorbic acid/100 g of pulp of Cupuaçu and 8–20 mg of dehydroascorbic acid/100 g of pulp of Cupuaçu was found. The sum of these contents results in 33–55 mg of total vitamin C/100 mg of pulp which is comparable to other fruits well-known for being good suppliers of this vitamin (lemon, *Citrus limon*, 46 mg of total vitamin C/100 g of raw juice; orange, *Citrus aurantium var dulcis*, 50 mg of total vitamin C/100 g of raw juice and melon, *Curcumis melo*, 42.2 mg of total vitamin C/100 g pulp) (Young & How, 1986).

3.2. Kinetics of ascorbic acid thermal degradation

From Fig. 1 it can be concluded that the thermal degradation reaction of ascorbic acid clearly deviates from simple first order leveling off after a certain time at a dimensionless concentration approximately equal to 0.4. Due to this fact a reversible first order model Eq. (1) was attempted to model the data, (Levenspiel, 1972).

$$\frac{C_{AA} - C_{AA_\infty}}{C_{AA_0} - C_{AA_\infty}} = e^{-k_1 t} \quad (1)$$

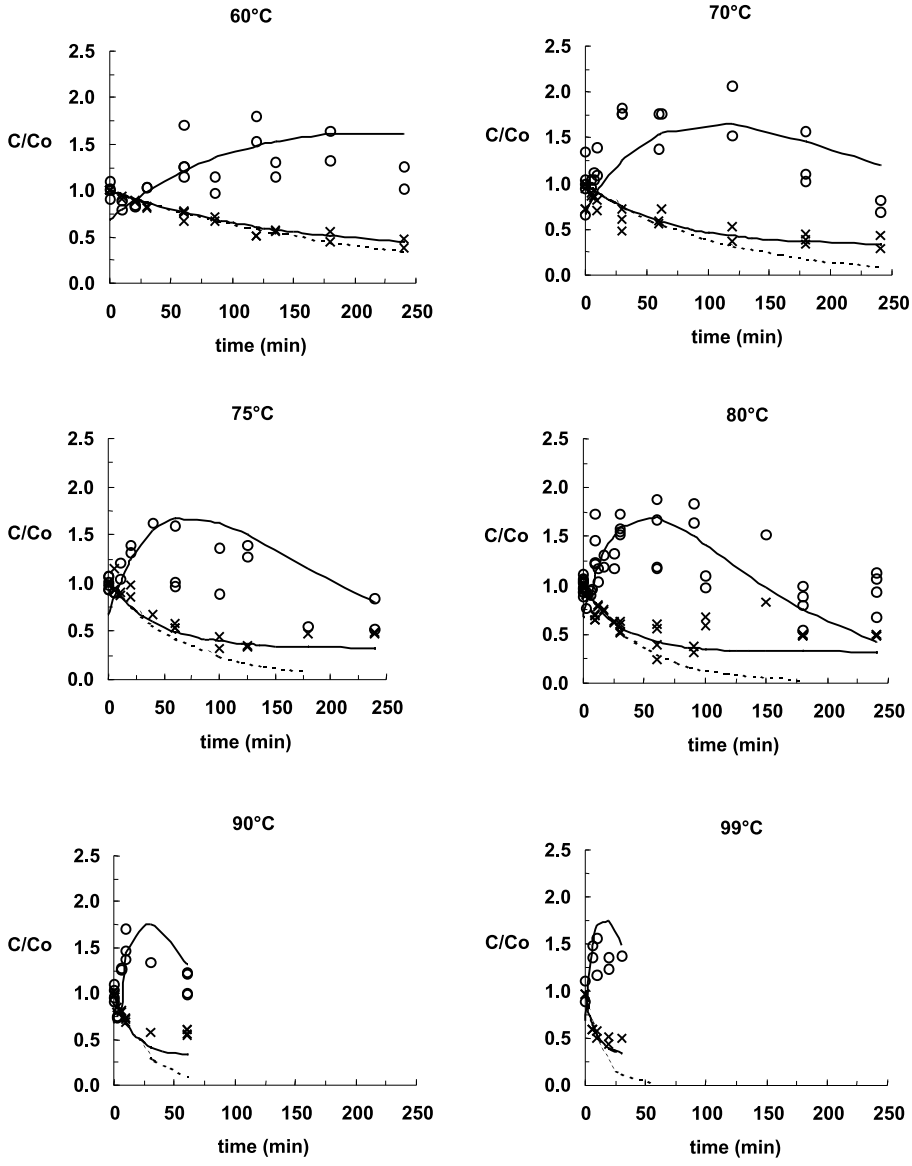

Fig. 1. Effect of temperature and time on the degradation of ascorbic acid (AA) (×) and dehydroascorbic acid (DHAA) (○) in Cupuaçu nectar (pH 3.2 and 18°Brix). Predicted values by the reversible first order kinetic model (lower line) and by the first order kinetic model (dashed line) for AA and predicted values by the mechanistic model (upper line) for DHAA.

where C_{AA} is the relative concentration of ascorbic acid (AA) at time t, C_{AA_0} the AA initial relative concentration ($\cong$1), C_{AA_∞} the AA relative concentration at time ∞ and k_1 is the rate constant.

It was also verified that the reaction rate constant temperature dependence followed the Arrehnius law:

$$k_1 = k_{1_{80^\circ C}} \exp\left\{ -\frac{Ea_1}{R}\left(\frac{1}{T} - \frac{1}{273+80}\right)\right\} \qquad (2)$$

where k_1 is the rate constant at the absolute temperature T, $k_{1_{80^\circ C}}$ the AA rate constant at the reference temperature 80°C, Ea_1 the AA activation energy and R is the universal gas constant (8.314 kJ/mol K). Estimated kinetic parameters using the software STATA are presented in Table 2 and the predicted data are represented by continuous lines in Fig. 1. The activation energy Ea_1 is in agreement with published data for other fruit juices (Table 1).

If only the first 30 min of thermal treatment are considered (most pasteurization processes nowadays are high temperature short time (HTST)), then a first order reaction (Eq. (3)) will present a good fit (Fig. 1 dashed lines) and the kinetic data are also presented in Table 2.

$$\frac{C_{AA}}{C_{AA_0}} = e^{-k_1 t} \qquad (3)$$

From Fig. 1 it can be observed that the deviation from simple first order for AA degradation is dependent on

Table 2
Kinetic parameters of thermal degradation of AA in Cupuaçu nectar (18°Brix, pH 3.2)

Fitted model	Cupuaçu nectar 18°Brix, pH 3.2			
	Ea_1 (kJ/mol)	$k_{1_{80°C}}$ (min^{-1})	C_{AA_∞}	R^2
$\frac{C_{AA} - C_{AA_\infty}}{C_{AA_0} - C_{AA_\infty}} = e^{-k_1 t}$	74 ± 5	0.032 ± 0.003	0.32	0.994
$\frac{C_{AA}}{C_{AA_0}} = e^{-k_1 t}$	73 ± 7	0.020 ± 0.001	0.00	0.996

temperature, starting deviating after around 1 h at 60°C but taking less than 30 min at 99°C. As the samples were heated for a longer period (up to 240 min) and the TDT tubes were only screw capped (not sealed), most probably, throughout the experiment, the dissolved oxygen reacted or escaped from the nectar leaving an anaerobic environment behind. Under anaerobic conditions the rate of degradation of ascorbic acid is reduced to a half or a third of the rate of the oxidative reaction (Gregory, 1996), and so, after a certain time, AA oxidation to DHAA became imperceptible reaching an equilibrium level. Therefore, AA behaved as a limiting reactant preventing the reaction to proceed to completion.

3.3. Kinetics of dehydroascorbic acid thermal degradation

A reversible equilibrium is well-known to occur between AA and DHAA Eq. (4), (Tannenbaum, 1976),

$$AA \underset{k_{(-1)}}{\overset{k_{(1)}}{\rightleftharpoons}} DHAA \xrightarrow{k_2} DKGA \tag{4}$$

DHAA is reduced to AA in the body or in the presence of reducing agents, such as SH_2, or of enzymes such as dehydroascorbate reductase or ascorbate free radical reductase present in animal tissue. In the present case, as there is no information available about the presence in the nectar of reducing agents, an irreversible consecutive reaction might be considered as the predominant reaction from AA to DHAA. The deketogulonic acid (DKGA) is the next product to be formed (Gregory, 1996) once the lactone bridge of DHAA is very susceptible of hydrolysis, even at low pH.

If the concentration of ascorbic acid (AA) is measured by $C^*_{AA} = C_{AA} - C_{AA_\infty}$, a reaction treated as reversible can be considered as irreversible and the conversion measured as a fraction of the maximum attainable (Levenspiel, 1972). Eq. (1) can then be treated as simple first order and consequently approximate the overall mechanism to two consecutive irreversible reactions:

$$C^*_{AA} \xrightarrow{k_1} C_{DHAA} \xrightarrow{k_2} C_{DKGA} \tag{5}$$

$$C^*_{AA} = e^{-k_1 t} C^*_{AA_0} \tag{6}$$

Hence, the way how DHAA varies with time can be expressed mathematically by Villota and Hawkes (1992)

$$\frac{dC_{DHAA}}{dt} = k_1 C^*_{AA} - k_2 C_{DHAA} \tag{7}$$

or

$$\frac{dC_{DHAA}}{dt} + k_2 C_{DHAA} = k_1 (C^*_{AA_0}) e^{-k_1 t} \tag{8}$$

This is a first order linear differential equation. By integrating with the initial condition $C_{DHAA} = C_{DHAA_0}$ at $t = 0$, the following analytical solution is obtained:

$$\frac{C_{DHAA}}{C_{DHAA_0}} = \left\{ \left[\left(C^*_{AA} / C^*_{AA_0} \right)^{k_2/k_1} \left(- C^*_{AA_0} / C_{DHAA_0} + ((k_2/k_1) - 1) \right) \right] + C^*_{AA} / C_{DHAA_0} \right\} / ((k_2/k_1) - 1) \tag{9}$$

where k_1 is given by Eq. (1) and replacing the kinetic parameters by the values already determined (Table 2). The reference temperature T_{ref} was considered to be the average temperature of the experiments (80°C) and k_2 is also assumed to be temperature dependent and to follow the Arrehnius law.

$$k_2 = k_{2_{80°C}} \exp\left\{ -\frac{Ea_2}{R} \left(\frac{1}{T} - \frac{1}{273 + 80} \right) \right\} \tag{10}$$

The concentration of ascorbic acid, C^*_{AA}, is given by Eq. (6).

The mechanistic model developed in this study, and described above, is in agreement with the oxidative pathway described before.

In Fig. 1 the upper points present the time evolution of dehydroascorbic acid (DHAA) concentration as a function of temperature. A one step non-linear regression was performed to all data (Arabshahi & Lund, 1985) using the model in Eq. (9) and the software STATA (Statacorp, 1995). The estimated kinetic parameters, $k_{2_{80°C}}$ and Ea_2, are presented in Table 3. Predicted values for DHAA concentration are also shown in Fig. 1 as continuous lines (upper line). The rate constant $k_{2_{80°C}}$ for the reaction of degradation of DHAA to form DKGA presents a small value in comparison

Table 3
Kinetic parameters of thermal degradation of DHAA in Cupuaçu nectar

Cupuaçu nectar 18°Brix, pH 3.2	
Ea_2 (kJ/mol)	65 ± 9
$k_{2_{80^\circ C}}$ (min^{-1})	0.013 ± 0.003
C_{DHAA_0}	0.27 ± 0.05
R^2	0.94

with the rate constant $k_{1_{80^\circ C}}$ of the reversible reaction between AA and DHAA. This indicates that during thermal processing the conversion of AA into DHAA is much more prevalent, confirming that the DHAA conversion into DKGA is more perceptive only when AA reaches the equilibrium concentration.

The experimental data of DHAA present some scattering mainly due to the fact that in the analytical method used the DHAA concentration was measured indirectly, and since the derivatization reaction of DHAA to DFQ has to be time and temperature controlled in the dark, any slight deviation from the optimal conditions (Zapata & Dufour, 1992) leads to large errors. This can explain the relatively low R^2 value (Table 3).

With the model developed the quantity of dehydroascorbic acid in the Cupuaçu nectar can be predicted with some accuracy, indicating whether the product is still protected against browning or not. The model developed may also be useful to attempt the modeling of the degradation of this form of vitamin C in other fruit juices or nectars.

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