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Reversible degradation kinetics of ascorbic acid under reducing and oxidizing conditions

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Abstract

Ascorbic acid and dehydroascorbic acid kinetics in model solutions, under oxidizing and reducing conditions, were determined using a first order reversible consecutive reaction model. The time-dependent changes for both ascorbic acid and dehydroascorbic acid were strongly correlated with the model described here. Presence of reducing or oxidizing agents in the medium affected the reaction rate constants. In control, the rate constant of reversible reaction in which dehydroascorbic acid reduces to ascorbic acid (k_2) was found to be almost zero, whereas the rate constants for oxidation of ascorbic acid to dehydroascorbic acid (k_1) and hydrolysis of dehydroascorbic acid to 2,3-diketogluconic acid (k_3) were close to each other. Addition of cysteine, as the reducing agent, into the medium significantly increased (k_2). Increasing the cysteine concentration also increased (k_2). Nevertheless, addition of Fe^{3+} as the oxidizing agent, into the medium had no effect on (k_2), but significantly increased k_1 and k_3.

Keywords: Ascorbic acid; Dehydroascorbic acid; Reversible degradation kinetic; Oxidation; Reduction

1. Introduction

Vitamin C is one of the most important nutritional quality factors in foods and has many biological activities in the human body. Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of ascorbic acid (AA; 2-oxo-l-threo-hexono-1,4, lactone-2,3 enediol). AA is the principal biologically active form but dehydroascorbic acid (DHAA; threo-2,3-hexodiulosonic acid-γ-lactone), an oxidation product, also exhibits biological activity (Lee & Kader, 2000; Wills, Wimalasiri, & Greenfield, 1984).

In food systems, the current effort is to develop and apply a systematic kinetic and modelling approach to the main quality indices of each product. By establishing the appropriate quality function, that describes the time–temperature dependence of the selected mode of degradation, a quantitative tool for shelf life estimation and management is obtained (Giannakourou & Taoukis, 2003). Sensitivity of AA under several process conditions and its nutritional benefits make it a good quality indicator. Therefore it is important to determine the behaviour of AA under several conditions.

It has been well known that a reversible equilibrium occurs between AA and DHAA, in which DHAA irreversibly hydrolyzes to 2,3-diketogluconic acid (DKGA) under certain conditions. Presence and amounts of oxidizing and reducing agents in the medium determine the reaction rate. In food systems, various oxidizing and reducing agents are naturally present, while some additional species may also occur during thermal treatment. Therefore, the reversible nature of this reaction should be taken into account for an accurate kinetic characterization of AA and DHAA. However, information about the reversible degradation of AA is lacking in the literature.

This paper describes a kinetic model for reversible consecutive reaction of AA to DHAA, with subsequent

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hydrolysis of DHAA to DKGA. Experimental data obtained under various reducing and oxidizing conditions fit the mathematical model well.

2. Material and methods

2.1. Materials

AA (min 99.7%), cysteine (min 99%), Fe(III)-nitrate (1000 mg/l, as iron standard solution) were obtained from Merck and dithiothreitol (DTT, min 99%) was obtained from Sigma. Deionized water was used in all experiments.

2.2. Preparation of model solutions

Three types of model solutions were used in order to determine the oxidation kinetics of AA under control, oxidizing and reducing conditions. The control was the aqueous solution of AA at a concentration of 200 mg/l. Second and third types of solutions were the aqueous mixtures of AA (200 mg/l) with Fe^{3+} (2 and 20 mg/l), and AA (200 mg/l) with cysteine(2 and 20 mg/l), respectively. In order to prevent the time-lag needed to increase the temperature of reaction medium, all reactions were started by introducing appropriate amounts of AA, cysteine and Fe^{3+} into water maintained at 90 °C to obtain final desired concentrations of each reagents in the reaction medium. The changes of AA and DHAA concentrations were monitored up to ca. 6 h.

2.3. Measurement of AA and DHAA

AA and DHAA analyses were performed according to Gökmen, Kahraman, Demir, and Acar (2000). The samples were divided into two parts: one part was directly analyzed for AA content. DTT was added to the other part at a concentration of 1 mg/ml and it was kept dark for 90 min to convert any DHAA to AA. After complete conversion of DHAA was achieved, the sample was analyzed for its total AA content. DHAA content of the sample was evaluated by the subtraction of first part's AA content from the second part's total AA content.

2.4. Derivation of mathematical model for reversible consecutive reaction kinetics

Fig. 1 depicts the reversible equilibrium which is well known to occur between AA and DHAA, in which DHAA irreversibly hydrolyzes to DKGA.

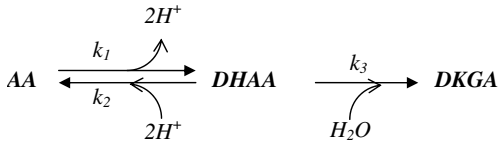

Fig. 1. Chemical reaction mechanism for the degradation of AA and DHAA.

The rates of AA and DHAA may be written as given below according to the reaction scheme shown in Fig. 1,

$$\frac{d[AA]}{dt} = -k_1[AA] + k_2[DHAA] \quad (1)$$

$$\frac{d[DHAA]}{dt} = k_1[AA] - k_2[DHAA] - k_3[DHAA] \quad (2)$$

Eqs. (1) and (2) may be rewritten in Laplace transformation in s-domain which yields Eqs. (3) and (4), respectively.

$$sAA(s) - AA(0) = -k_1AA(s) + k_2DHAA(s) \quad (3)$$

$$sDHAA(s) - DHAA(0) = k_1AA(s) - (k_2 + k_3)DHAA(s) \quad (4)$$

Rearranging Eq. (4) gives Eq. (5)

$$DHAA(s) = \frac{k_1AA(s) + DHAA_{(0)}}{s + k_2 + k_3} \quad (5)$$

Combining Eqs. (5) and (3) gives Eq. (6)

$$AA(s) = \frac{AA_{(0)}[s + k_2 + k_3] + k_2DHAA_{(0)}}{s^2 + s(k_1 + k_2 + k_3) + k_1k_3} \quad (6)$$

Roots of the denominator (a and b) of Eq. (6) may be calculated as

$$a = \frac{-(k_1 + k_2 + k_3) + \sqrt{(k_1 + k_2 + k_3)^2 - 4k_1k_3}}{2} \quad (7)$$

$$b = \frac{-(k_1 + k_2 + k_3) - \sqrt{(k_1 + k_2 + k_3)^2 - 4k_1k_3}}{2} \quad (8)$$

Eq. (6) may now be rewritten as

$$AA(s) = \frac{k_2(AA_{(0)} + DHAA_{(0)}) + k_3AA_{(0)}}{(s - a)(s - b)} + \frac{sAA_{(0)}}{(s - a)(s - b)} \quad (9)$$

Inverse Laplace of Eq. (9) yields Eq. (10) in t-domain.

$$AA = \frac{(k_2 + k_3)AA_{(0)} + k_2DHAA_{(0)}}{(a - b)}(e^{at} - e^{bt}) + \frac{AA_{(0)}}{(a - b)}(ae^{at} - be^{bt}) \quad (10)$$

Rearranging Eq. (10) gives Eq. (11),

$$AA = \left(\frac{(k_2 + k_3 + a)AA_{(0)} + k_2DHAA_{(0)}}{(a - b)}\right)e^{at} - \left(\frac{(k_2 + k_3 + b)AA_{(0)} + k_2DHAA_{(0)}}{(a - b)}\right)e^{bt} \quad (11)$$

and defining m_{AA}, n_{AA}, p_{AA}, and q_{AA} as

$$m_{AA} = \left(\frac{(k_2 + k_3 + a)AA_{(0)} + k_2DHAA_{(0)}}{(a - b)}\right)$$

$$n_{AA} = \left(\frac{(k_2 + k_3 + b)AA_{(0)} + k_2DHAA_{(0)}}{(a - b)}\right)$$

$$p_{AA} = a$$

$$q_{AA} = b$$

Eq. (11) becomes

$$AA = (m_{AA} * \exp(p_{AA} * t)) - (n_{AA} * \exp(q_{AA} * t)) \quad (12)$$

which was used for AA kinetics in this study Rearranging Eqs. (5) and (6) for DHAA yields Eq. (13)

$$DHAA(s) = \frac{k_1 AA_{(0)}[s + k_2 + k_3] + k_1 k_2 DHAA_{(0)}}{(s^2 + s(k_1 + k_2 + k_3) + k_1 k_3)(s + k_2 + k_3)} + \frac{DHAA_{(0)}}{s + k_2 + k_3} \quad (13)$$

Defining $c = -(k_2 + k_3)$and combining into Eq. (13) gives Eq. (14)

$$DHAA(s) = \frac{k_1(k_2 + k_3)AA_{(0)} + k_1 k_2 DHAA_{(0)}}{(s-a)(s-b)(s-c)} + \frac{k_1 s AA_{(0)}}{(s-a)(s-b)(s-c)} + \frac{DHAA_{(0)}}{(s-c)} \quad (14)$$

Inverse Laplace of Eq. (14) yields Eq. (15) in t-domain

$$DHAA = \frac{(k_1 AA_{(0)} a + \varepsilon)}{(a-b)(a-c)} e^{at} - \frac{(k_1 AA_{(0)} b + \varepsilon)}{(a-b)(b-c)} e^{bt} + \left[\frac{(k_1 AA_{(0)} c + \varepsilon)}{(a-c)(b-c)} + DHAA_{(0)}\right] e^{ct} \quad (15)$$

where ε is defined as $\varepsilon = k_1 k_2 DHAA_{(0)} + AA_{(0)} k_1 (k_2 + k_3)$. Since $\left[\frac{(k_1 AA_{(0)} c + \varepsilon)}{(a-c)(b-c)} + DHAA_{(0)}\right] = 0$ in Eq. (15), it may be rewritten as

$$DHAA = \frac{(k_1 AA_{(0)} a + \varepsilon)}{(a-b)(a-c)} e^{at} - \frac{(k_1 AA_{(0)} b + \varepsilon)}{(a-b)(b-c)} e^{bt} \quad (16)$$

Defining m_{DHAA}, n_{DHAA}, p_{DHAA}, and q_{DHAA} as

$$m_{DHAA} = \frac{(k_1 AA_{(0)} a + \varepsilon)}{(a-b)(a-c)}$$

$$n_{DHAA} = \frac{(k_1 AA_{(0)} b + \varepsilon)}{(a-b)(b-c)}$$

$$p_{DHAA} = a$$

$$q_{DHAA} = b$$

Eq. (16) becomes

$$DHAA = (m_{DHAA} * \exp(p_{DHAA} * t)) - (n_{DHAA} * \exp(q_{DHAA} * t)) \quad (17)$$

which was used for DHAA kinetics in this study.

3. Results and discussion

AA is known to be thermolabile. To date, several authors have studied its thermal degradation kinetics in foods during thermal treatments and have reported that it follows a first order reaction model (Frias & Oliveira, 2001; Frias, Oliveira, Cunha, & Oliveira, 1998; Giannakourou & Taoukis, 2003; Johnson, Braddock, & Chen, 1995; Saguy, Kopelman, & Mizrah, 1978; Uddin, Hawlader, Ding, & Mujumdar, 2002; Vieira, Teixeira, & Silva, 2001). The AA degradation mechanism is specific to a particular system, as it depends on several factors (Tannenbaum, 1976). Work on the infuence of pH, oxygen (Eison-Perchonok & Downes, 1982), metal ions (Hsieh & Harris, 1987), sucrose (Hsieh & Harris, 1987, 1993), enzymes and amino acids (Jung, Kim, & Kim, 1995) on the rate of AA degradation has contributed to a better knowledge of the behaviour of AA in different systems.

However, limited information is available on the kinetics of AA and DHAA degradations from the mechanistic point of view. In this study, AA and DHAA kinetics in model solutions under oxidizing and reducing conditions were determined using a first order reversible model. Time-dependent changes of AA and DHAA concentrations were monitored at 90 °C. Time versus concentration data were analyzed according to Eqs. (12) and (17) to determine the model parameters (n, m, p, q) for AA and DHAA kinetics using Curve Expert version 1.3 software. The rate constants were then calculated accordingly. Although the initial concentration of DHAA ($DHAA_0$) was zero in our experiments, the data analyses were performed assuming it to be close to zero (10^{-10} mg/l) in order to prevent the iteration process failing.

Figs. 2 and 3 show time versus concentration plots of AA and DHAA, respectively. The time-dependent changes for both AA and DHAA were strongly correlated with the model described here. The results of non-linear regression analyses are summarized in Tables 1 and 2 for AA and DHAA, respectively.

The loss of AA was found to be 72.5% in control medium after 6 h at 90 °C. The addition of cysteine to the reaction medium decreased, while the addition of Fe^{3+} increased, the loss of AA. Overall losses of AA were found to be 33.5% and 22.5% after 6 h at 90 °C in the presence of 2 mg/l and 20 mg/l of cysteine, respectively. Contrarily, overall losses of AA were found to be 84.8% and 93.2% after 6 h at 90 °C in the presence of 2 mg/l and 20 mg/l of Fe^{3+}, respectively.

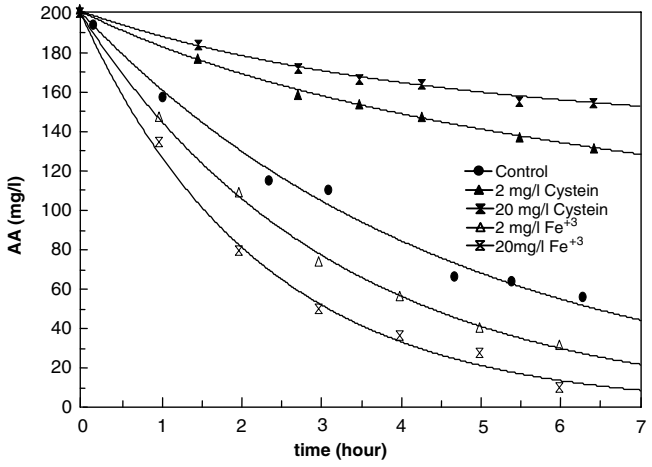

Fig. 2. Change of AA concentrations under various oxidizing and reducing conditions at 90 °C (solid lines indicate model fit).

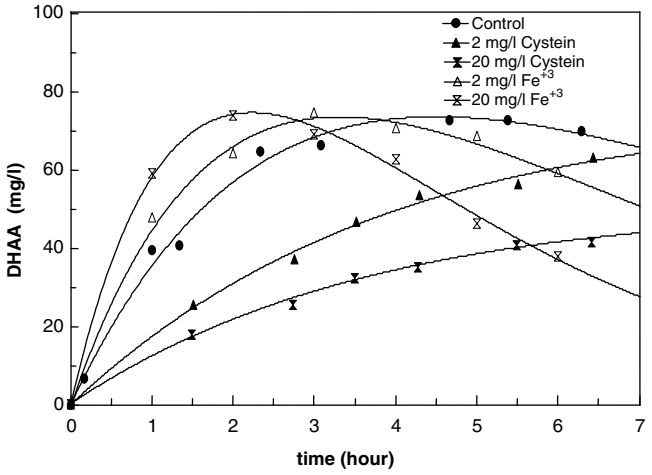

Fig. 3. Change of DHAA concentrations under various oxidizing and reducing conditions at 90 °C (solid lines indicate model fit).

Table 1
Model parameters estimated for AA according to Eq. (12) under various reducing and oxidizing conditions at 90 °C

	m_{AA}	p_{AA}	n_{AA}	q_{AA}	r^2
Control	199.8	−0.2144	−0.8	−0.2143	0.9931
2 mg/l Cysteine	77.4	−0.2325	−122.7	−0.0120	0.9982
20 mg/l Cysteine	50.3	−0.2478	−149.8	−0.0077	0.9965
2 mg/l Fe^{3+}	200.1	−0.3145	−0.8	−0.3122	0.9987
20 mg/l Fe^{3+}	200.0	−0.4485	−1.3	−0.4476	0.9972

Table 2
Model parameters estimated for DHAA according to Eq. (17) under various reducing and oxidizing conditions at 90 °C

	m_{DHAA}	p_{DHAA}	n_{DHAA}	q_{DHAA}	r^2
Control	538336.8	−0.2213	538336.2	−0.2214	0.9930
2 mg/l Cysteine	85.4	−0.0095	85.3	−0.2393	0.9954
20 mg/l Cysteine	56.5	−0.0090	56.4	−0.2555	0.9970
2 mg/l Fe^{3+}	26426.6	−0.3005	26425.8	−0.3027	0.9950
20 mg/l Fe^{3+}	104766.5	−0.4489	104766.2	−0.4498	0.9932

The reaction rate constants calculated from the model parameters given in Tables 1 and 2 are summarized in Table 3. Presence of reducing or oxidizing agents in the medium affected the reaction rate constants. In control, the rate constant of reversible reaction, in which DHAA reduces to AA (k_2), was found to be almost zero, expectedly. The rate constants for oxidation of AA to DHAA (k_1) and hydrolysis of DHAA to DKGA (k_3) were close to each other in control. Addition of cysteine, as the reducing agent, to the medium significantly increased (k_2). Increasing the cysteine concentration also increased (k_2). Nevertheless, addition of Fe^{3+} as the oxidizing agent, to the medium had no effect on k_2, but significantly increased k_1 and k_3.

Vieira, Teixeira, and Silva (2000) have also studied the thermal degradation kinetics of vitamin C in cupuaçu nectar. As there is no information available about the presence of reducing agents, such as SH_2, dehydroascorbate reductase or ascorbate free radical reductase in this nectar, authors have assumed that an irreversible consecutive reaction might be considered as the predominant reaction from AA to DHAA, and reduced the overall mechanism to two consecutive irreversible reactions. However, this approach cannot be used unless the absence of reducing agents has been confirmed in the medium.

The mathematical model presented here estimates the rate constants, using the reaction mechanism shown in Fig. 1 in which the reversible nature of AA degradation is taken into account. As shown in Table 4, calculated (k_1) values do not differ for either reversible or irreversible approaches in the absence of reducing agent in the reaction medium. The differences between the calculated (k_1) values for reversible and irreversible models were determined to be less than 2% for the reaction performed with AA only, and with AA and Fe^{3+}. However, calculated (k_1) values significantly differed from each other for reversible and irreversible models in the presence of cysteine. Increased reduction potential also increased the absolute difference between (k_1) values calculated for the two models. Although non-linear regression analyses of these two models yield reasonable regression coefficients ($R^2 > 0.98$) for both reversible and irreversible models, the calculated

Table 4
Calculated mean (k_1) values for reversible and irreversible models

	k_1 (h^{-1})		Absolute difference (%)
	Reversible	Irreversible[a]	
Control	0.218	0.214	1.74
2 mg/l Cysteine	0.0977	0.0639	34.6
20 mg/l Cysteine	0.0682	0.0395	42.5
2 mg/l Fe^{3+}	0.309	0.315	1.95
20 mg/l Fe^{3+}	0.449	0.449	0.10

[a] k_1 is calculated by fitting the experimental data to the model of $[AA] = [AA]_0 \cdot \exp(-k_1 t)$ derived for irreversible first order consecutive reaction of AA → DHAA → DKGA.

Table 3
Calculated reaction rate constants for AA and DHAA under various reducing and oxidizing conditions at 90 °C

	k_1 (h^{-1})	k_2 (h^{-1})	k_3 (h^{-1})
Control	$0.218 \pm 4.66 \times 10^{-3}$	$0.249 \times 10^{-8} \pm 6.34 \times 10^{-7}$	$0.218 \pm 5.35 \times 10^{-3}$
2 mg/l Cysteine	$0.0977 \pm 5.33 \times 10^{-4}$	$0.123 \pm 0.642 \times 10^{-5}$	$0.0260 \pm 3.92 \times 10^{-3}$
20 mg/l Cysteine	$0.0682 \pm 1.05 \times 10^{-3}$	$0.161 \pm 0.164 \times 10^{-4}$	$0.0286 \pm 3.68 \times 10^{-3}$
2 mg/l Fe^{3+}	$0.309 \pm 8.51 \times 10^{-3}$	$0.184 \times 10^{-8} \pm 0.115 \times 10^{-8}$	$0.306 \pm 8.10 \times 10^{-3}$
20 mg/l Fe^{3+}	$0.449 \pm 6.30 \times 10^{-4}$	$0.212 \times 10^{-8} \pm 0.285 \times 10^{-8}$	$0.449 \pm 1.19 \times 10^{-3}$

(k_1) was not true for the reaction performed under reducing conditions.

The results indicate that vitamin C is retained in its AA form and its decomposition via reversible oxidation of AA to DHAA and irreversible hydrolysis of DHAA to DKGA is limited during thermal treatment if sufficient reducing agent is present in the medium. However, AA readily oxidizes to DHAA if an oxidizing agent is present in the reaction medium.

Half-life of AA was estimated to be 3.21 h for control medium at 90 °C. It increased to 18.0 h and 52.5 h in the presence of 2 mg/l and 20 mg/l of cysteine, respectively, while it decreased to 2.22 h and 1.56 h in the presence of 2 mg/l and 20 mg/l of Fe^{3+}, respectively.

4. Conclusion

This paper describes a kinetic model for reversible oxidation of AA to DHAA with subsequent hydrolysis of DHAA to DKGA. The model successfully fits the experimental data obtained for control, as also for strong reducing and oxidizing conditions at 90 °C. The results clearly show that the oxidation–reduction (OR) potential of the reaction medium significantly affect the rate of AA oxidation. These findings suggest that the OR potential of foods determines the rate of AA oxidation. However, the exact mechanism and in particular, the reversible nature of AA oxidation, should be taken into account to estimate the kinetic constants more accurately.

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