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# Kinetics of ascorbic acid loss during thermal treatment in different pH buffer solutions and the presence of oxygen

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#### **Abstract**

Ascorbic acid stability is greatly influenced by temperature, oxygen content. Both exposures to oxygen and prolonged heating in the presence of oxygen destroy ascorbic acid. Thermal degradation kinetics of ascorbic acid in sodium acetate buffer (0.2 M, pH 5.0) and sodium phosphate buffer (0.1 M, pH 7.0) were studied at the temperature range of 80 to 100°C and the different molar ratio between oxygen and ascorbic acid. The obtained results showed that the decrease in AA concentration as a function of time at constant temperature occurred in two phases. The biphasic model was used to describe the loss of AA in aqueous solution due to thermal treatment. The AA degradation had occurred during thermal processing probably due to oxidation (aerobic degradation). When the oxygen was totally used up, the anaerobic degradation dominated and took place much more slowly than aerobic degradation. At low AA concentration (≈0.28 mM) and an oxygen concentration of 0.25 mM, most of the degradation of AA occurred under aerobic conditions, however, AA was further degraded through an anaerobic pathway at a higher ratio of AA to oxygen concentration [≈0.57:0.25 (mM:mM) and 1.42:0.25 (mM:mM)]. The estimated activated energy E<sub>a</sub> values, the temperature sensitivity of the kvalues was lower at pH 7.0 than at pH 5.0. Studying the degradation of ascorbic in a model system is essential in order to fully understood and applied effectively in food products processing.

#### 1. Introduction

Vitamin C (C<sub>6</sub>H<sub>8</sub>O<sub>6</sub> or ascorbic acid) belongs to the water-soluble vitamins. Vitamin C is one of the principal nutrients in citrus fruits and is mainly found in its reduced form, L-ascorbic acid (AA). However, its oxidized form, L-dehydroascorbic acid (DHAA) is reported to possess equivalent biological activity to AA (Kall, 2003; Martí, 2009). AA is an important vitamin in the human diet and is abundant in plant tissues. Vitamin C is needed for the growth and repair of tissues in all parts of the body and to form collagen, an important protein used to make skin, scar tissue, tendons, ligaments and blood vessels. Vitamin C is essential for the healing of wounds and for the repair and maintenance of cartilage, bones and teeth (Walingo, 2005). AA is extensively used in the food industry, not only for its nutritional value but also for its functional contribution to product quality. For example, AA can improve the color and palatability of many kinds of food products because of its role as an antioxidant.

Fruit and vegetables are the major sources of vitamin C in human diets. It has been reported that ascorbic acid is thermolabile and it may be degraded during food processing. The concentration of AA can also decrease during storage, depending on the storage conditions, such as temperature, oxygen content, pH and light (Manso et al., 2001; Ottaway, 2002). Processing stability of ascorbic acid in model systems or in food systems can be affected by several intrinsic (chemical structure, pH, oxygen) and extrinsic (temperature) factors. Information on vitamin C stability both in buffer solutions and in food matrices is available (Viera et al., 2001). The results on the stability and the degradation kinetics of AA during processing are still contradictory (Rojas and Gerschenson, 2001; Blasco et al., 2004). In order to evaluate the process impact on vitamin C stability, the AA degradation in the model system was studied under isothermal conditions on a kinetic basis. Moreover, the influence of pH and molar ratio between oxygen and AA on AA stability was evaluated.

#### 2. Materials and methods

#### 2.1. AA sample preparation

AA [Molarmass (MM)=176.13 g, Merck, Darmstadt, Germany] was used in this research. For each experiment, the working solutions were daily prepared by dissolving AA (50, 100, 250 and 500 mg/mL) in thermostated (25°C) acetate buffer (0.2 M, pH 5.0) or phosphate buffer (0.1 M, pH 7.0). After sample preparation and prior to the thermal treatments, the working solutions with different AA concentrations were flushed with humidified air (flow rate =350 cc min<sup>-1</sup>) for 20 min at 25°C to achieve approximately 8.0 ppm ( $\approx 0.25$ mM) soluble oxygen in the samples. After flushing, the oxygen concentration was measured (Strathkelvin Instruments oxygen meter model 782, Scotland). The samples were enclosed in glass vials with a rubber septum (800 mL, 30 mm length, 8.2 mm diameter, Cleanpack, Belgium) or in 0.3 mL flexible microtubes for thermal treatments. All procedures of sample preparation and treatments were carried out under subdued light (by covering the samples with aluminum foil) and avoided from direct contact with air.

#### 2.2. Isothermal treatment

Thermal experiments were performed in a water bath  $(T<90^{\circ}C)$  or in an oil bath  $(T\geq90^{\circ}C)$ . To ensure isothermal heating, the sample solutions were filled in capillary tubes (Hirchmann, 1.15 mm i. d. x 150 mm length). After preset time intervals, the capillaries were withdrawn from the water bath or the oil bath and immediately cooled in ice water (below 4 degrees celsius) to stop the thermal degradation. The samples were stored in ice water until the HPLC assay. The blank  $(C_o)$  was defined as the concentration of the untreated sample. Each heat treatment was performed twice.

### 2.3. Data analysis

In literature, the degradation kinetics of AA is mostly described by first-order model (Karhan *et al.*, 2004; Özkan *et al.*, 2004; Sapei and Hwa, 2014). Under isothermal conditions, the degradation rate constant k could be estimated by equation 1.

$$C_t = C_0 \exp(-kt) \tag{1}$$

Where  $C_t$  is the residual concentration of AA at time t (mM);  $C_o$  is the initial concentration of AA (mM); k is the degradation rate constant and t is the treatment time (min).

In the presence of oxygen, it is assumed that AA degrades through aerobic and anaerobic pathways, each reaction follows first-order kinetics. Therefore, equation 1 can be modified to equation 2.

$$C_t = C_a \exp(-k_a t) + C_{an} \exp(-k_{an} t)$$
 (2)

Where  $C_a$  indicates the proportion of the aerobic degradation (% or mM);  $C_{an}$  the proportion of the anaerobic degradation (% or mM);  $k_a$  the aerobic degradation rate constant and  $k_{an}$  the anaerobic degradation rate constant. These kinetic parameters can be estimated using non-linear regression analysis (SAS, 2001).

The temperature dependence of the degradation rate constant (k) is estimated as activation energy  $(E_a)$  using the Arrhenius equation (equation 3).

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

Where k is the degradation rate constant (min<sup>-1</sup>),  $k_{ref}$  is the degradation rate constant at  $T_{ref}$ ,  $E_a$  is the activation energy (kJ/mol),  $R_T$  is the universal gas constant (8.314 Jmol<sup>-1</sup>K<sup>-1</sup>), T is the absolute temperature (K) and  $T_{ref}$  is the absolute reference temperature (K). When the natural logarithm of the degradation rate constant is plotted as a function of reciprocal of absolute temperature at constant pressure (equation 4), the activation energy can be derived from the slope based on linear regression analysis.

$$\ln(k) = \ln(k_{ref}) + \left\lceil \frac{E_a}{R_T} \left( \frac{1}{T_{ref}} - \frac{1}{T} \right) \right\rceil$$
 (4)

2.4. HPLC analysis for AA identification and quantification

HPLC (1200 Series Agilent Technology) equipment using a RP-C<sub>18</sub> column (Eclipse XDB-C<sub>18</sub>, 5µm, 4.6 x 150 mm, Agilent Technologies, Germany) and Chemstation software was used to identify and quantify AA. The column temperature was maintained at 25°C. AA was detected using a UV detector at 254 nm. The eluent was a mixture of acetic acid buffer (10 mM, pH 3.0) and 1 mM Na<sub>2</sub>EDTA with a flow rate of 0.4 mL/ min, followed by Jedlička and Klimeš (2004) with some modifications. The sample injection volume was 20 µL. The AA peak was found at a retention time between 2.7 to 3.0 mins after injection. The concentration of AA was calculated based on peak area in comparison to the external standard solutions of the same component. The correlation coefficient (r<sup>2</sup>) of the standard curves in this study was above 0.97.

#### 3. Results and discussion

3.1 Effect of molar ratio between oxygen and AA on thermal stability of AA

The effect of molar ratio between oxygen and AA on the vitamin stability in acetate buffer (0.2 M, pH 5.0) and

phosphate buffer (0.1 M, pH 7.0) during thermal treatment was studied. The initial concentration of dissolved oxygen in the sample was ≈0.25 mM and the concentrations of AA were 50 mg/mL (≈0.28 mM), 100 mg/mL ( $\approx 0.57$  mM), 250 mg/mL ( $\approx 1.42$  mM) and 500 mg/mL (≈2.84 mM). The decrease in AA concentration as a function of time at constant temperature occurred in two phases, illustrated for observations at 80°C (Figure 1). At an AA concentration of approximately 0.28 mM and an oxygen concentration of 0.25 mM, most of the degradation of AA occurred under aerobic conditions. At a higher ratio of AA to oxygen concentration [approximately 0.57:0.25 (mM:mM) and 1.42:0.25 (mM:mM)], AA was further degraded through an anaerobic pathway. Similar degradation behavior was observed at other treatment temperatures and pH values.

The thermal stability of AA in all systems was investigated at various constant temperatures from 80 to 100°C. As expected, increasing temperature accelerates degradation (Figure 2). To estimate the kinetic parameters, the biphasic model (equation 2) was used. The correlation between the predicted and the experimental AA concentration was satisfactory (Figure 3).

Table 1 showed that the degradation rate constant increases with increasing temperatures (above  $80^{\circ}$ C), but different treatment temperatures do not influence the proportion of AA degraded under aerobic ( $C_a$  in mM)

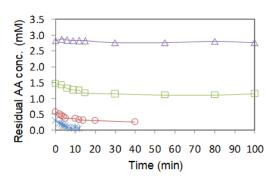


Figure 1. The residual AA concentration as a function of treatment time at different AA concentration  $\approx$ 0.28 mM (\*);  $\approx$ 0.57mM (o);  $\approx$ 1.42mM ( $\square$ );  $\approx$ 2.84 mM ( $\Delta$ ) [in phosphate buffer (0.1 M, pH 7.0) at 80°C with 0.25 mM O<sub>2</sub> conc.]

and anaerobic conditions ( $C_{an}$  in mM). When oxygen is completely consumed, AA anaerobic degradation occurs and this anaerobic degradation occurs more slowly than the AA aerobic degradation. For some kinetic parameters, the standard errors of the estimated values are relatively large probably due to limited data points to describe both the aerobic and anaerobic phase. In order to achieve higher accuracy of the estimated kinetic parameters, experiments for longer treatment times might be required. At a higher ratio of AA to oxygen concentration (AA concentration of 250 µg/mL ≈1.42 mM or 500  $\mu$ g/mL  $\approx$ 2.84 mM and in presence of 0.25 mM oxygen), the portion of the aerobic degradation at the beginning of the kinetic study was less pronounced as compared to the anaerobic degradation. It suggests that the degradation of AA occurs mainly through an anaerobic pathway. Our results are in good agreement with the studies of Roig et al. (1995) This evidence clarifies why some authors have reported first-order kinetics for AA degradation (Viera et al., 2000; Rojas and Gerschenson, 2001; Giannakourou and Taoukis, 2003; Blasco et al., 2004, Karhan et al., 2004), while others have described AA degradation using a biphasic model due to the aerobic and anaerobic degradation pathways (Wilson et al., 1995; Abbasi and Niakousari, 2008). Lavelli and Giovanelli (2003) reported pseudofirst-order kinetics of ascorbic acid degradation in tomato products. Manso et al. (2001) have applied a Weibull model to describe the kinetics of AA degradation. According to Gregory (1996) and Gibbons

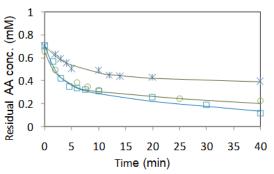


Figure 2. The residual AA concentration as a function of treatment time at different temperature 80°C (\*), 90°C (o) and 100°C ( $\square$ ) [AA concentration  $\approx$ 0.57 mM in phosphate buffer (0.1 M, pH 7.0) with 0.25 mM O<sub>2</sub> conc.]

Table 1. Effect of temperature on AA (100  $\mu$ g/mL  $\approx$  0.57 mM) stability in phosphate buffer (0.1 M, pH 7.0°)

T (°C)	$k_a  (\text{min}^{-1})$	$k_{an} (x10^{-3} \text{min}^{-1})$	$C_a$ (%)	$C_a (\mathrm{mM})^{\mathrm{b}}$	$C_{an}(\%)$	$C_{an}$ (mM)
80	$0.20{\pm}0.08^{c}$	$3.69\pm3.87$	36.29±7.23	$0.25 \pm 0.05$	64.07±7.38	$0.45 \pm 0.05$
90	$0.41\pm0.11$	12.90±4.22	47.53±5.97	$0.31\pm0.04$	52.89±5.26	$0.34 \pm 0.03$
100	$0.45 \pm 0.12$	24.20±5.49	50.33±6.18	$0.35 \pm 0.04$	50.34±5.58	$0.36 \pm 0.04$
$E_a$ (kJ/mol)	$44.4 \pm 18.8^d$	103.2±18.0				
	$r^2 = 0.85$	$r^2 = 0.97$	/	/	/	/

<sup>&</sup>lt;sup>a</sup>The initial oxygen concentration was  $\approx$  0.25 mM, <sup>b</sup>Calculated using MM of AA of 176.13 Da, <sup>c</sup>Asymptotic standard error of non linear regression, <sup>d</sup>Standard error of linear regression.

et al. (2001), AA is converted to DHAA in presence of oxygen. This reaction can be reversed by a strong reducing agent such as dithiothreitol (DTT). DHAA degrades further via the hydrolysis to 2,3-diketogulonic acid. Under anaerobic conditions, AA degrades via several steps to furfural instead of DHAA (Rodriguez et al., 1991). Yuan and Chen (1998) have reported that no DHAA is significantly formed under anaerobic conditions but AA degrades due to the cleavage of the ring and the addition of water, afterwards the decarboxylation and the intermolecular rearrangement and finally dehydrations to form furfural. In the presence of oxygen, AA is oxidized yielding DHAA and one mole AA consumes one mole of oxygen (equation 5)

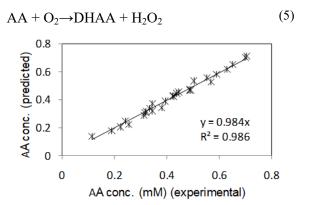


Figure 3. Correlation between the experimentally determined concentration values and the estimated concentration values for AA (≈0.57 mM) degradation in phosphate buffer (0.1 M, pH 7.0) at different temperatures (80 to 100°C) using the biphasic model (equation 2)

Ottaway (2002) found that the concentration of aerobically degraded AA was approximately 2 times as high as the initial molar oxygen concentration. These data suggest the decomposition of  $H_2O_2$  to  $H_2O$  and  $O_2$ . In this study, the estimated  $C_a$  values were situated approximately 0.3 mM which is close to the initial oxygen concentration of 0.25 mM. Temperature dependency of the rate constants for AA degradation in the temperature range studied could be described by the Arrhenius equation (equation 4). Comparing the  $E_a$  values, the temperature sensitivity of the k-values for the degradation of AA under aerobic conditions is lower than that under anaerobic conditions (Table 1). This value is in line with the values previously.

#### 3.2 Effect of pH

pH can influence the thermostability of AA. The maximal thermostability of AA has been reported between pH 4 and 6 (Moser and Bendich, 1990). Cooking losses of AA depend on the intensity of heating, leaching as a consequence of surface area exposed to water, oxygen, pH and the presence of transition metals (Naidu, 2003). As discussed previously (ratio of AA to oxygen concentration ≈0.57:0.25 mM), the thermal

degradation of AA occurred mainly under aerobic conditions due to oxidation. AA aerobic degradation at pH 7.0 was faster than at pH 5.0 under aerobic condition. However, the degradation of AA is retarded at higher pH values under anaerobic condition (Figure 4). At higher AA oxygen ratio (≈ [1.42:0.25] and [2.84:0.25]), AA degradation occurred mainly under anaerobic conditions (Figure 5). Coker *et al.* (1993) found that increasing pH from 0.5 to 11 reduces the anaerobic degradation of vitamin C whereas a maximum aerobic degradation of AA was found at higher pH value.

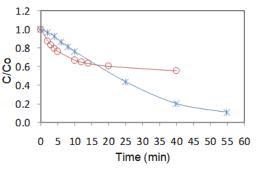


Figure 4. The ratio of AA concentration to initial concentration as a function of treatment time [AA  $\approx 0.57$  mM [in acetate buffer (0.2 M, pH 5.0) (\*) and phosphate buffer (0.1 M, pH 7.0) (o) at 80°C with 0.25 mM O<sub>2</sub> conc.]

From the thermodynamic point of view, the oxidation is favored in alkaline media due to the decrease in ascorbic acid redox potential (Manuel de Villena Rueda et al., 1989). The oxidation process is impeded in acidic environment and at low temperature. Comparing the estimated  $E_a$  values, the temperature sensitivity of the k-values was lower at pH 7.0 than at pH 5.0 (Table 2). Loss in AA contents under different pH conditions was investigated. Results revealed that after 55 mins of heat treatment, the percentage of AA losses were found 7.66% and 2.69%, respectively in pH 5 and 7. The mechanism of anaerobic degradation of AA is not fully understood. It is assumed that AA can be anaerobically degraded due to a direct cleavage of the 1,4-lactone bridge. The opening of the lactone ring is favored at low pH values (pH 3-4) (Gregory, 1996). It can be observed that the AA degradation depends on oxygen levels and pH. Similar results on the effect of pH on anaerobic degradation are found in the literature. Wedzicha (1984) reported that the rate of AA degradation was pH dependent and shows a maximum degradation rate at pH 5. Rojas and Gerschenson (2001) mentioned that the optimum for AA degradation was at pH lower than 5.5 (for AA anaerobic degradation in the aqueous model system). Vieira et al. (2000) reported that the lactone bridge of DHAA was very susceptible to hydrolysis, even at low pH and Ottaway (2002) indicated that the rate of AA degradation in aqueous solutions was pH-dependent with a maximum rate at about pH 4. AA degradation under acidic conditions occurs according to

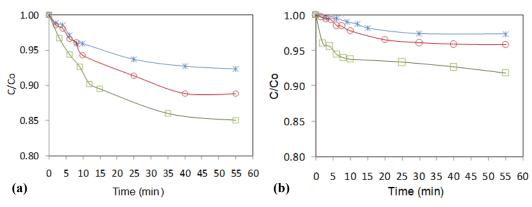


Figure 5. The residual AA concentration as a function of treatment time at different temperature 80°C (\*), 90°C (o) and 100°C ( $\square$ ) [AA concentration  $\approx$  2.84 mM in **a.** acetate buffer (0.2 M, pH 5.0) and **b.** phosphate buffer (0.1 M, pH 7.0) with O<sub>2</sub> conc.]

Table 2. Effect of pH on AA (≈1.42 mM) stability in acetate buffer (0.2 M, pH 5.0<sup>a</sup>) and phosphate buffer (0.1 M, pH 7.0<sup>a</sup>)

T (°C)	$k_a  (\text{min}^{-1})^*$	<i>C<sub>a</sub></i> (%)	$C_a  (\mathrm{mM})^{\mathrm{b}}$	C <sub>an</sub> (%)	Can (mM)			
Acetate buffer (0.2 M, pH 5.0)								
80	$0.03\pm0.02$	$4.68\pm8.92$	$0.08\pm0.15^{c}$	95.25±8.69	1.55±0.14			
90	90 $0.09\pm0.03$		$0.20\pm0.05$	86.58±3.88	$1.24 \pm 0.06$			
100	$0.44\pm0.17$	23.95±3.47	$0.38\pm0.05$	76.05±2.26	$1.19\pm0.04$			
$E_a$ (kJ/mol)	$155.27 \pm 11.75^{d}$ $r^{2} = 0.99$	nd	nd	nd	nd			
Phosphate buffer (0.1 M, pH 7.0)								
80	$0.08 \pm 0.02$	$23.79\pm3.04$	$0.42 \pm 0.05$	77.08±3.12	$1.36 \pm 0.06$			
90	$0.25 \pm 0.21$	16.01±6.50	$0.25\pm0.10$	85.81±5.89	$1.32\pm0.09$			
100	$0.38\pm0.09$	17.58±1.87	$0.30\pm0.03$	82.29±1.74	1.39±0.03			
$E_a$ (kJ/mol)	$87.38 \pm 22.20$ $r^2 = 0.94$	nd	nd	nd	nd			

<sup>a</sup>The initial oxygen concentration was ≈ 0.25 mM, <sup>b</sup>Calculated using MM of AA of 176.13 Da, <sup>c</sup>Asymptotic standard error of non linear regression, <sup>d</sup>Standard error of linear regression, nd: not determined,  $*k_{an}$  could not be properly estimated due to limited data set

two reaction pathways (Yuan and Chen, 1998; Abbasi and Niakousari, 2008). Under hydrogen-ion-catalyzed aerobic conditions, AA is converted to 2-furoic acid and 3-hydroxy-2-pyrone via DHAA and under anaerobic condition, AA directly degrades to furfural. However, high pH values do not favor the formation of furfural, 2-furoic acid and 3-hydroxy-2-pyrone, but high pH values could promote the production of an unknown compound.

#### 4. Conclusion

The concentration of dissolved oxygen has an important influence on AA stability. Therefore, decreasing oxygen concentration is needed to protect AA during processing. Degradation of AA in aqueous solution due to heat treatment was observed and a biphasic model is adequate to describe the time-dependent changes due to thermal treatment. When the oxygen was totally used up, the anaerobic degradation dominated and took place much more slowly than aerobic degradation. It is found that increasing temperature enhances the degradation of AA. At all temperature treatments, pH had a different effect on the

aerobic and anaerobic degradation of AA. These obtained results are important for the development of technology for ascorbic acid-rich foods. In addition, the kinetics of vitamin C degradation must be coupled with the destruction kinetics of microbial and other spoilage agents for process optimization, leading to achieving a safe food product with high nutritional values.

#### **Conflict of interest**

The authors declare no conflict of interest.

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