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ORIGINAL ARTICLE

An Investigation into the Degradation of Ascorbic Acid in Solutions

Claire D. Munialo[a,b] and Vassilis Kontogiorgos[a*]

[a]Department of Chemical and Biological Sciences, University of Huddersfield, Queensgate, Huddersfield HD1 3DH, UK.
[b]Physics and Physical Chemistry of Foods, Department of Agro-technology and Food Sciences, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, the Netherlands.

[*]Corresponding Author:

Claire Munialo

Email: Claire.munialo@wur.nl

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Abstract

The kinetics of the degradation of ascorbic acid (AA) in solution during heating at 30, 50, 70 and 90°C for 180 min and during 7 weeks of storage at 4°C and 20°C were investigated. The concentration of AA in solution was determined by the fluorimetric method before heating and storing the samples under different conditions. The retention of AA after heating at 30, 50, 70, and 90°C and storage at 4°C and 20°C was 87.6, 84.7, 73.8 and 43.6% (for heated samples) and 74.3 and 77.7% (for stored samples) respectively. Overall, there was a significant correlation ($p<0.05$) between the loss of AA as a result of heating of the samples or the storage of the samples at 4°C or 20°C. The degradation of AA in aqueous solution at each temperature was shown to follow a first-order kinetic model. The temperature-dependence of degradation was adequately modelled by the Arrhenius equation. The activation energy was 6.06 kcal/mole (for samples subjected to heat treatment) and 1.30 kcal/mole (for samples stored at 4°C and 20°C). The Q_{10} values were found to be 1.3 (for samples subjected to heat treatment) and 1.1 (for samples stored at 4°C and 20°C). The results from this study show that AA is a labile nutrient that can be easily lost during storage or processing. The results from this study contribute to the enhancement of the knowledge of the parameters that are of significance in the degradation of AA and thus provide an understanding of the conditions that can be used to optimize heat processing and storage of liquid foods that are rich in AA such as fruit juices.

Keywords: Ascorbic acid, temperature-dependence, stability, degradation, fluorimetric method.

Introduction

Vitamin C (ascorbic acid) is known to be one of the most unstable nutrients found in food (Moreira *et al.,* 2006). Several studies have demonstrated the rate of ascorbic acid (AA) degradation in various foods (Sinha *et al.,* 2012). Thermal degradation of AA has been reported to be one of the many routes of vitamin C loss from foods (Castro *et al.,* 2004). The substantial loss of AA has also been reported to occur via the mechanism of leaching given that AA is highly soluble in water (McKillop *et al.,* 2002).

Several methods such as titration with dichlorophenolindophenol (DCPIP) dye, high performance liquid chromatography, spectrophotometric, fluorimetric and turbidimetry have been used for the analysis of AA (Rodriguez *et al.,* 1992). In all mentioned methods of AA analysis, the fluorimetric method was reported to be the most accurate as it measures both AA and dehydroascorbic acid, which are the active forms of vitamin C (Nakajima *et al.,* 2009).

Several authors have reported instability of AA during storage and thermal processing. Studies on process optimization of foods have reported AA to be more sensitive to thermal degradation in natural products than in buffered model systems (Oliveira, 1998). L-ascorbic acid was shown to be more sensitive to heat in oranges than in tomatoes (Van den Broeck *et al.,* 1998), whereas the storage temperature was shown to be the most important factor that determines the stability of AA against degradation (Roig *et al.,* 1995). Low temperature storage was reported to be imperative in order to study L-ascorbic acid decay (Roig *et al.,* 1995). Furthermore, L-ascorbic acid was found to be

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significantly more stable in the modified matrix of pre-treated samples compared to that of untreated and blanched samples (Dermesonlouoglou *et al.,* 2007). Saturated solutions of two vitamin C forms were reported to differ in pH, given that vitamin C is more susceptible to degradation at a higher pH (Hiatt *et al.,* 2010). Contrarily, the retention of AA in blood orange juice showed a linear reduction in concentration with time (Choi *et al.,* 2002), whereas a much better retention of AA was found in pasteurized orange juice samples which were stored at 4 °C as compared to those at 25 °C (Zerdin *et al.,* 2003). However, the study of the stability of AA in solution over a long storage time and at varying temperature ranges has not been fully investigated. The aim of this study was to investigate the interaction between time and temperature in the degradation of AA in solution and to model it using first principles of chemical kinetics. This study aims at contributing to the knowledge on the conditions that result in the degradation of AA and thus provide possible insights on storage conditions that are important in the enhancement of the stability of AA in aqueous solutions against degradation.

Materials and methods

Materials

AnalaR® grade (99.9% purity) crystalline ascorbic acid (AA) was obtained from British Drug House (BDH) International limited (Poole, England). Metaphosphoric acid (MPA), sodium acetate trihydrate, and glacial acetic acid were all purchased from Fisher (Leicestershire, UK). *O*-phenylenediamine dihydrochloride (OPD) was purchased from Sigma-Aldrich (Dorset, UK). Crystalline boric acid was obtained from Acros organics, (Geel, Belgium). All reagents were of analytical grade and used without further purification. All solutions were prepared with MilliQ water.

Ascorbic acid (AA) determination

The analysis of AA was carried out using the fluorimetric method as previously described (AOAC, 2006). In summary, 2 ml AA standard solution was added to 8 ml MPA and treated with activated carbon (activated charcoal- DARCO G-60) in the ratio of 50:1.The samples were placed in 2 ml test tubes and 5 ml OPD solution added to each tube. The tubes were then properly mixed (Rotamixer Deluxe, Hook and Tucker instruments Ltd, UK) and kept in the dark for 35 min, after which the fluorescence was compared on a Jenway 6280 fluorimeter (Bibby Scientific Ltd, Staffordshire, England) with an excitation wavelength of 350 nm and an emission wavelength of 430 nm as previously described (Nielsen, 2010).

The monitoring of the stability of AA against temperature degradation was carried out following the incubation of AA solutions at different temperatures (30, 50, 70 and 90 °C). Freshly prepared AA solutions were incubated at each temperature for up to180 min. Subsequently, 10 ml of standard AA was transferred into seven 15 ml test tubes and heated to the desired temperature in a water bath.

To investigate the stability of AA following storage, freshly prepared AA was stored at room (20°C) and refrigerated (4°C) temperature. A fluorimetric test was performed on the standard samples on the day of preparation after which the samples were divided into 2 equal portions. One sample was stored in the refrigerator at 4°C, whereas the other sample was stored at room temperature (20°C) for a period of 7 weeks and the AA concentration was determined weekly using the method for AA determination described above. Triplicate relative fluorescence readings of each standard were recorded at an emission wavelength of 430 nm with an excitation wavelength of 350 nm using a Jenway 6280 fluorimeter (Bibby Scientific Ltd, Staffordshire, England).

Subsequently, the recorded data (both for samples that had been heat treated and those stored at 20°C and 4°C) were analysed statistically to determine any significant differences between the AA concentration following the heat treatment or storage. The dependence of the degradation rate constant (k_T) on temperature was quantified by the Arrhenius equation as expressed below (Holme *et al.,* 2010).

$$k_T = A_O exp\left(-\frac{E_a}{RT}\right)(1)$$

Where, k_T is the rate constant, E_a is activation energy of the reaction (kJ mole^{-1}), R is the universal gas constant (8.3145 kJ mole^{-1}K^{-1}), T is absolute temperature (K), and A_0 is the frequency factor, also referred to as a pre-exponential constant (s^{-1}). Knowing the rate constant k_T, the activation energy (E_a) was calculated.

Statistical analysis

The statistical Package for Social Sciences (SPSS) software version 17.0® (SPSS Inc., International Business Machines, Chicago, USA) was used to analyse the result for significant differences whereas Microsoft Excel software (Microsoft Corporation, Redmond, USA) was used for regression analysis as previously described (Nisha *et al.,* 2004).

Results and Discussion

The retention of vitamin C (ascorbic acid, AA) has often been used as an estimate of the overall

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nutrient retention in food products as it is by far the most unstable nutrient (Kiremire *et al.,* 2010). To investigate the stability of AA at varying conditions, AA solutions were prepared as described in section 2.2, followed by the application of a heat treatment to the solutions or storing at varying temperatures and the changes in AA concentration as well as the stability of AA were recorded over time.

The stability of AA against temperature induced degradation

The stability of AA in solution following heating of the samples was determined as a function of time. Fig 1 show a combined graph for AA concentration following the heating of AA solutions to 30°C, 50°C, 70°C and 90°C for up to 180 min.

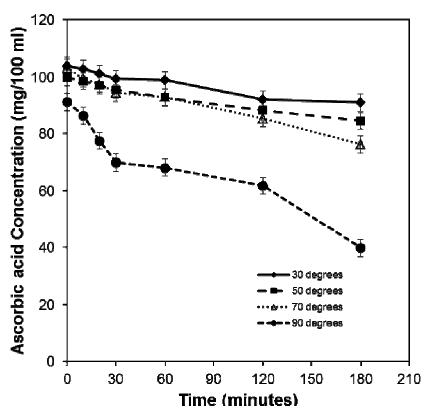

Fig 1: Changes AA concentration over heating time of up to 180 min at 30°C, 50°C, 70°C and 90°C.

From Fig 1, the stability of AA in solutions that were heated at lower temperatures was shown to be higher compared to heating of the solutions at higher temperatures. The determined AA concentration were higher following the heating of AA solution at 30°C than heating of AA at 50°C, 70°C and 90°C. From the statistical analyses performed on AA degradation data, paired sample t-test showed no significant difference between temperature and time ($p>0.05$) in relation to the degradation of AA (Results not shown). Conversely, a paired sample correlation of the stability of AA against degradation as a result of heat treatment for up to 180 min showed a significant difference in the relationship between temperature and heating time in their contributions to the degradation of AA ($p<0.05$) (Table 2). The initial AA concentration in the solution before heating of AA solutions to 30, 50, 70 and 90°C was 103.8, 99.8, 103.1 and 91.2 mg/100ml respectively. Following the heat treatment, the AA concentration decreased to 90.9, 84.5, 76.1 and 39.8 mg/100 ml for solutions heated at 30, 50, 70 and 90 °C respectively. The final AA concentration decreased with increasing temperature and heating duration which coincided with the reduction in retention of AA (%) in the samples which varied from 87.6% for solutions heated at 30°C to 43.6 % for solutions heated at 90°C.

The half-life of AA which is the time required for AA to degrade to 50 % of its original value was calculated from the rate constant as 0.693/k. The half-life was found to be higher (about 14 h) for a sample heated to 30°C compared to those heated to other temperatures (Table 1). This was clearly seen from k = 0.0008, 0.001, 0.0017 and 0.0044 min^{-1} obtained from the kinetic plots for heating the sample to 30, 50, 70 and 90°C respectively (Table 1). Following heating of the samples, the rate constants for AA degradation increased from 0.0008 min^{-1} for 30°C to 0.0044 min^{-1} for 90°C and the half-life decreased from 14 h to about 3 h for an increase in heating temperature from 30 to 90°C (Table 1). Rate constants in orange juice have been reported to range from 0.00105 to 0.03471 min^{-1} for a temperature range of 70-98 °C (Johnson *et al.,* 1995). Thus, the rate constants obtained for AA in solution heated at 50, 70 and 90 °C in the present study were in the range of the reported rate constants (Johnson *et al.,* 1995). High temperature has been reported to favour the breakdown of vitamin C whereas the AA concentration has been shown to decrease during storage, drying, heating, and oxidation of food (Morris *et al.,* 2004). However, studies on the kinetics of heat/enzymic degradation of AA in fluted pumpkin leaves reported that there were relatively higher degradation rates observed in puree system for pumpkin leaves at lower temperature (60°C), whereas at the higher temperatures (90°C) that cause enzyme inactivation, AA became more stable (Ariahu *et al.,* 1997).

Further, analysis of AA stability against heating by applying statistical analyses to the obtained data showed a correlation coefficient of >-0.90 in all cases which suggests that the degradation of AA in solution as a result of the heat treatment follows a first order reaction for the studied temperature ranges (Results not shown). The changes in AA in the forms of *ln C/CO* with heating AA solutions for up to 180 min at different conditions are as shown in Fig 3. The results also confirm the findings that the degradation of AA in solution follows the first order kinetics. When the obtained data was analysed using the standard integrated rate equation of linear regression to determine the overall order and rate constant for the degradation reactions, negative correlation coefficient were obtained at all heating temperatures (Results not shown). The results confirm that AA degradation occurred during heating of the solutions at different -

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Table 1: Kinetic parameters for AA degradation in solution during heating and storage

Parameter	Temperature (°C)	1/Kelvin (k^{-1})	Rate (min^{-1})	ln (Rate)	Half-life
AA	4 (277 °K)	$3.6x10^{-3}$	0.0419	-7.13	14.32 weeks
AA	20 (295°K)	$3.4x10^{-3}$	0.0484	-6.91	16.54 weeks
AA	30 (303 °K)	$3.3x10^{-3}$	0.0008	-6.38	14.44 h
AA	50 (323°K)	$3.13x10^{-3}$	0.001	-5.53	11.55 h
AA	70 (343°K)	$2.93x10^{-3}$	0.0017	-3.17	6.80 h
AA	90 (363°K)	$2.83x10^{-3}$	0.0044	-3.03	2.63 h

Table 2: Paired samples correlations for samples stored at 4°C and 20°C as well as those subjected to heat treatment for up to 180 min at 30°C, 50°C, 70°C, and 90°C.

		N	Correlation	Significant difference
Pair 1	4 °C & 20 °C	7	0.981	0.000
Pair 2	AA at 30 °C & Time	7	-.963	.000
Pair 3	AA at 50 °C & Time	7	-.982	.000
Pair 4	AA at 70 °C & Time	7	-.987	.000
Pair 5	AA at 90 °C & Time	7	-.951	.001

temperatures. These findings show that AA is not stable and hence will always degrade. The results from this study are in agreement with the study of Cruz and co-workers who found vitamin C to decompose under unfavourable conditions such as high temperature (Cruz *et al.,* 2008). The results from the present study show that both time and temperature have a significant effect on AA concentration. Therefore, both time and temperature must be taken into account when considering AA stability during cooking, thermal processing or in the prediction of nutrient quality loss during storage.

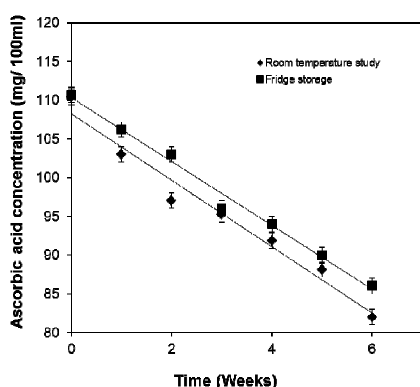

Fig 2: Changes in AA concentration over storage time at room and temperature.

The stability of AA in relation to storage conditions

Previous studies have reported storage duration and condition to be an important parameter in vitamin C degradation (Morris *et al.,* 2004; Lee and Nagy, 1988). To investigate the effect of storage conditions on the AA degradation, AA solutions were stored at room (20°C) and refrigerated (4°C) conditions for up to 7 weeks. The results for changes in AA concentration in relation to storage time are shown in figure 2. The initial AA concentration for samples stored at 20°C was 110.4 mg/ml, whereas the concentration of samples stored at 4°C was 110.8 mg/ml during the first week of storage. The AA concentration reduced to 82 mg/ml for samples stored at 20°C and 86 mg/ml for samples stored at 4°C when the samples were analysed at the end of the 7th week. Statistical analyses of the storage data showed a significant difference between storing AA solution under 20°C and 4°C ($p<0.05$) (Table 2). Paired sample t-test performed on data from samples stored at 20°C and 4°C confirmed a significant difference in the AA concentration for samples stored at 20°C when compared to those stored at 4°C ($p<0.05$) (Results not shown). This shows that the storage conditions have a reductive effect on AA concentration. Comparing the results of samples stored at 20°C to those stored at 4°C, degradation was shown to occur in both samples but the rate was slower at 4°C than 20°C as expected (Fig 2). This was confirmed from the rate constant of 0.0484 min^{-1} (for samples stored at 20°C) and 0.0419 min^{-1} (for samples stored at 4°C) (Table 1). The highest rate constant was 0.0484

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min^{-1} for samples stored at 20°C indicating that AA degradation was fastest in this sample. The obtained rate constants in this study following the storage of AA under varying conditions for up to 7 weeks of storage were close to the previously reported rate constant of 0.0444 min^{-1} for thermally processed sonicated orange juice during storage at 10 °C (Tiwari *et al.,* 2009).

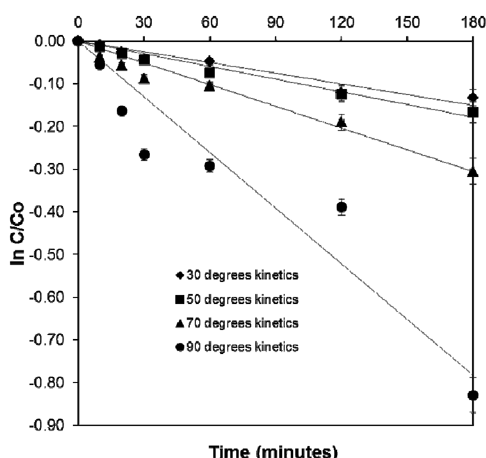

Fig 3: Degradation kinetics of AA solution during heating time of up to 180 min at 30°C 50°C 70°C and 90°C.

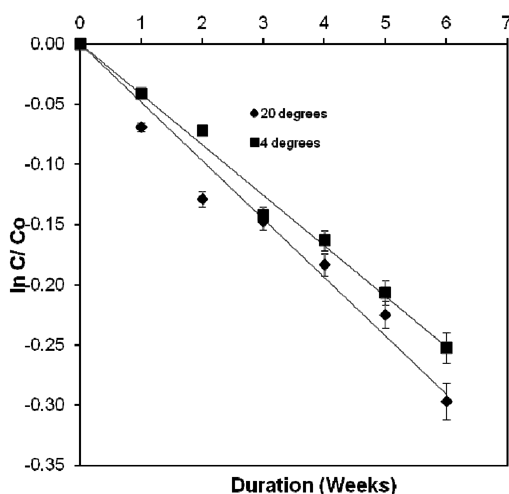

Fig 4: Degradation kinetics of AA solution during storage for up to 7 weeks at room (20°C) and refrigerated storage (4°C).

The changes in AA in the forms of *ln C/CO* with storage for up to 7 weeks at different conditions are shown in figure 4. From Fig 4, the degradation of AA in solution during storage under various conditions was also shown to follow the first order kinetics similar to heating at various temperatures. The findings were in agreement with the study carried out by Tiwari and co-workers who showed that AA degradation in sonicated orange juice during storage followed first order kinetics (Tiwari *et al.,* 2009). Contrastingly, the degradation of AA in orange juice stored at refrigerated and room temperature was reported to follow zero order kinetics (Faramade, 2007). The reaction rate constant, *k* was determined for each temperature from the slope of the line obtained by least squares regression analysis for refrigerated (4°C) and room storage (4°C) samples. The *k* value showed that there was a decrease in the AA concentration over time. The curve was steeper for samples stored at 20°C than for sample stored at 4°C, indicating higher degradation in samples stored at 20°C (Fig 4). This also confirms temperature to be one of the factors affecting the stability of AA against degradation. A prediction of the time required for AA concentration to reduce to half the initial value showed the estimated half-life to be about 14 weeks (for samples stored at 20°C) and 17 weeks (for samples stored at 4°C) (Table 1). The results obtained from the present study are in agreement with a similar study on AA degradation in commercial orange juice which showed that the kinetics of AA degradation were faster at high temperature (Faramade, 2007). Likewise, increasing drying temperature led to higher degradation rates in tomatoes under different drying conditions (Marfil *et al.,* 2008). Contrarily, a rapid AA loss was found to occur at refrigerated temperature as opposed to room temperature (Kouniaki *et al.,* 2004). A marginally lower retention of AA in refrigerated samples of un-pasteurized Iranian lemon juice has also been reported (Abbasi and Niakousari, 2007). In contrast, the final AA concentration in unpasteurized sour orange juice in Iran was found to be approximately 20mg/100ml regardless of storage conditions (Amiri and Niakousari, 2008). The results from the present study show a very strong relationship between AA content and samples stored at 4°C over time (in weeks). Thus, storage temperature should be taken into consideration when considering AA stability during storage of foods rich in vitamin C.

Arrhenius plots

Using linear regression, the degradation data were analysed to determine an overall order and rate constant for the degradation reaction. From the kinetic plot for all temperatures, AA was shown to be more stable when the samples were heated at 30°C as compared to being heated at 90°C. The rate constants obtained from the combined kinetic plot for all temperatures were used in the preparation of an Arrhenius plot for temperature-dependence rate of AA

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degradation. Kinetic parameters for AA degradation are as shown in Table 1. There was a relatively strong relationship between natural logarithm of the rate constant and the absolute temperature in °K for heating. Subsequently, the frequency factor was calculated using the natural logarithm of 2.7394 and -0.809. The results showed values of 15.5 and 0.5 min^{-1} for temperature and time studies respectively. The activation energy (E_a) was also calculated as a product of the gas constant, R (1.987 cal M^{-1} K^{-1}) and the slope of the graph obtained by plotting *ln k* versus $1/T$. The activation energy values obtained were 25.4 (temperature study) and 5.4 kJ/mole (storage time study).

An empirical application of the Arrhenius model can be of significant practical value for shelf-life modelling and predictions (Koutsoumanis *et al.,* 2000). The temperature quotient (Q_{10}) values, which are the increase in reaction rates for a 10°C increase in temperature, were calculated for samples heated for up to 180 min at varying temperatures and for samples stored at 20°C and 4°C using the formula previously reported (Hobbie and Kahn, 1999). The Q_{10} values were found to be 1.3 (for heating at 30, 50, 70 and 90°C) and 1.1 (for samples stored at 20°C and 4°C).

Previous studies have reported a wide variation in activation energy (E_a) for AA degradation in different food systems. The E_a of vitamin C degradation during infra-red drying of apple slices was found to be 3.54 kcal/mole (Timoumi *et al.,* 2007). A study on the AA degradation kinetics in amla showed the E_a to be 4.09 for amla and 4.49 kcal/mole for vitamin solution (Nisha *et al.,* 2004). In the present study, E_a calculated for the temperature study was 6.06 kcal/mole. The findings were in good agreement with reported *Ea* values of 5–40 kcal/mole for AA (Villota and Hawkes, 2007; Uddin *et al.,* 2002). TheQ_{10} values were found to be 1.3 and 1.1 for temperature (at 30, 50, 70 and 90°C) and storage conditions study (refrigerated and room temperature). The Q_{10} value for temperature study was in strong agreement with Q_{10} value of 1.2 for AA degradation obtained from a similar study on the influence of simultaneous variations in temperature and relative humidity on the chemical stability of two vitamin C forms and implications for shelf-life models (Hiatt *et al.,* 2010). However, Q_{10} value for storage temperature (room and refrigerated) in the present study was lower than 3.1 at 45° Brix and 3.6 at 64° Brix in a study on the effects of temperature, solid content and pH on the stability of black carrot anthocyanins (Kirca *et al.,* 2007). The findings suggest that Arrhenius models can be effectively used to predict the shelf-life of processed foods that are rich in AA at varying temperature conditions which can result in the improvement of the keeping quality and nutritive value of such foods.

Conclusions

The kinetics of AA degradation in solution were investigated as a function of heating and storage time in order to model AA degradation using first principles of chemical kinetics. Heating conditions and duration of storage as well as temperature were shown to result in AA degradation. Heating of AA at low temperatures resulted in less degradation compared to higher temperatures. Storing of AA solutions at refrigerated temperature enhanced AA stability compared to room temperature. Degradation of AA was found to follow first order kinetics for both samples subjected to heat treatment as well as those stored under various conditions. The findings indicate that heating duration and temperature, as well as storage conditions contribute significantly to the stability of AA. Thus, heating and storage conditions should be taken in to consideration during heat processes of foods rich in AA in order to maintain the nutritive quality of such a labile nutrient.

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