A study on degradation kinetics of ascorbic acid in drumstick (*Moringa olifera*) leaves during cooking

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Abstract: The kinetics of ascorbic acid degradation in drumstick (*Moringa olifera*) leaves as well as in pure ascorbic acid solutions at the initial concentrations present in drumstick leaves over a temperature range of 50–120 °C (isothermal temperature process) has been studied. The degradation kinetics of ascorbic acid was also evaluated in normal open-pan cooking, pressure-cooking and a newly developed and patented fuel-efficient eco cooker (non-isothermal heating process). The ascorbic acid degradation followed first-order reaction kinetics where the rate constant increased with an increase in the temperature. The temperature dependence of degradation was adequately modelled by the Arrhenius equation. A mathematical model was developed using the isothermal kinetic parameters obtained to predict the losses of ascorbic acid from the time-temperature data of the non-isothermal heating/processing method. The results obtained indicate the ascorbic acid degradation is of similar order of magnitude in all the methods of cooking. © 2005 Society of Chemical Industry

Keywords: ascorbic acid degradation; kinetics; drumstick leaves; cookers

INTRODUCTION

Fruits and vegetables undergo various handling, storage and processing steps before they are consumed. Thermal processing gives microbial safety, texture and flavour, but the application of heat to vegetables causes the loss of vitamins and minerals. Water-soluble ascorbic acid is destroyed easily during cleaning and cooking.¹ The presence of ascorbic acid in processed food is considered to be associated with quality because of its relative instability to heat, oxygen and light.

Ascorbic acid is highly sensitive to various modes of processing. Factors that can influence the nature of the degradation mechanism include temperature, salt and sugar concentration, pH, oxygen, enzymes and metal catalyst.^{2–5} Kirk and coworkers conducted extensive studies of the degradation of the ascorbic acid during storage in different food systems.^{6–9} Immediate processing of leafy vegetables after harvesting or storage in a refrigerator conserves the ascorbic acid concentration.¹⁰ Some studies have been reported on the effect of γ -irradiation on ascorbic acid content during storage conditions, initially showed 6–15% additional loss of ascorbic acid compared with

the control but on prolonged storage, showed large losses compared with the control. γ -Irradiation did not result in any enhanced loss during cooking in comparison with the non-irradiated tubers.

Knowledge of degradation kinetics, including reaction order, rate constant and activation energy, is essential to predict quality loss during storage as well as thermal processing. Kinetic data are reported for the loss of ascorbic acid on storage of some fruit juices like orange juice,^{13,14} grapefruit juice and cocktails of orange, grapefruit, pineapple, apple, mango, kiwi and apricot juices in different proportions¹⁴ and of tomato juice.7 Several kinetic studies have also been conducted on the degradation of ascorbic acid in buffer solution.^{15,16} However very little work has been reported on kinetic data for thermal destruction of ascorbic acid in food systems. The stability of ascorbic acid in tomato juice as a function of temperature, pH and metal catalyst has been reported earlier.⁷ However, the kinetic data obtained from these studies at 7.2-37 °C will not be applicable to the higher temperatures of household cooking or industrial processing. Rates of ascorbic acid degradation during thermal processing of canned peas have been studied in which a temperature range of 110-132.2 °C has

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been covered.¹⁷ A study on the degradation of ascorbic acid in dried guava during storage reports ascorbic acid degradation to be a pseudo-first-order reaction.¹⁸ Pseudo-first-order degradation kinetics were reported for ascorbic acid during conventional and ohmic heating with activation energy of $12.6 \text{ kcal mol}^{-1}$ and $12.5 \text{ kcal mol}^{-1}$ for conventional and ohmic heating, respectively.¹⁹ Thermal degradation of green asparagus ascorbic acid, when heated between 110 and 140 °C, are reported to follow first-order kinetics, with an activation energy of $12.3 (\pm 2.0) \text{ kcal mol}^{-1}.^{20}$

Different processing methods are used in household cooking. Some of them are normal open-pan cooking, pressure-cooking, microwave cooking and gradual cooking using slow cookers. A fuel-efficient cooker has recently been developed in our institute.²¹ The principle of this cooker is based on multiple effect evaporation, slow heating proportional to pick-up rate of the supplied heat by the cooking vessel, and insulation, these features being combined in one unit. Not much work has been reported on the degradation kinetics of ascorbic acid with respect to different cooking methods.

Green leafy vegetables have long been recognized as an important source of ascorbic acid. A scan of the nutrient composition of different green leafy vegetable showed drumstick (*Moringa olifera*) leaves to be a rich source of ascorbic acid.^{22–24} The drumstick fruit is also a very good source of ascorbic acid. It is grown and consumed in the tropics and semi-arid tropics of the world. In India, these vegetables are, however, most often consumed when cooked, which leads to the losses in ascorbic acid. Hence, the leaves of drumstick, which is an important leafy vegetable in many part of India, were selected as a model food system for the studies.

The main objectives of this study were therefore to (1) to determine the kinetic parameters for ascorbic acid degradation in a model food system as well as in pure vitamin solutions (to study the effect of food components on degradation) over a temperature range of 50-120 °C (isothermal temperature), (2) to study the degradation kinetics of ascorbic acid in different cooking methods (non-isothermal process), (3) to develop a mathematical model relating the calculated kinetic data from the isothermal heat processing and time-temperature profiles of different cooking methods (non-isothermal process) and (4) to apply this model to predict the ascorbic acid degradation for non-isothermal heating processes from the time-temperature data of the non-isothermal heating process, and to compare it with the actual degradation values.

MATERIALS AND METHODS Materials

The drumstick leaves were collected fresh from the UICT gardens in sealed polyethylene bags. Ascorbic acid was obtained from M/S Himedia, Lab Pvt Ltd, Mumbai, India. All the other reagents used in the work

were obtained from SD Fine Chemicals, Mumbai, India. All the chemicals used were of AR grade.

Heat treatments

Heat treatments were carried out at different temperatures (50 60, 70, 80, 90, 100 and 120 °C) for 0-60 min. A water-bath was used as a heating device. (For 120 °C an autoclave was used). To study the degradation of ascorbic acid in pure solution, ascorbic acid concentration was taken to be the same as that of the initial concentration of the model system. Samples (10 g) were transferred into a 100-ml beaker containing 40 ml of distilled water and pre-heated to the particular temperature at which the studies were done. Samples, along with water, were withdrawn periodically and immediately analyzed for ascorbic acid.

Estimation of ascorbic acid

The heat-treated leafy vegetable samples, along with the water, were ground to a paste with minimum amount of 3% metaphosphoric acid in a mortar and pestle. This was then transferred into a 100-ml volumetric flask with the help of a funnel. The mortar and pestle were rinsed several times with small amounts of 3% metaphosphoric acid and added back to the volumetric flask. The volume was made up to 100 ml using 3% metaphosphoric acid, mixed thoroughly, and then filtered through filter paper. The filtrate (2 ml) was placed in a 50-ml stoppered conical flask followed by 2 ml of acetate buffer (pH 4.0, prepared by mixing 1 litre of 50% CH₃COONa·3H₂O with 1 litre of glacial acetic acid), 3 ml of Tillman's dye (2,6dichlorophenol-indophenol) and 15 ml of xylene. It was shaken vigorously for 10s and the solvent was allowed to separate. The lower water layer was pipetted out and the colour of the xylene layer was measured at 520 nm using a spectrophotometer²⁵ (Hitachi, Singapore, U-2001). All the experiments were done in triplicate.

Cooking studies

For cooking studies, normal open-pan cooking (20 min), pressure cooking (15 min) and cooking in a newly developed and patented fuel-efficient slow cooker, named 'Eco-cooker', (30 min on simmer and 30 min holding period) were selected as different cooking methods. The time and flow rates of fuel gas for the eco cooker was selected as per the instructions given for its usage.²¹ The same 1:4 (wt/vol) ratio of the leaves to water were taken and cooked using these three different cooking methods. Samples were withdrawn periodically, and analyzed for ascorbic acid as described earlier.

Time-temperature data

Time-temperature data for each of the cooking methods was monitored using a digital thermocouple.

Kinetic calculations

Kinetic calculations were done based on the following formulae:

A general reaction rate expression for degradation kinetics can be written:²⁶

$$-\mathbf{d}[C]/\mathbf{d}t = k[C]^m \tag{1}$$

where C is the quantitative value (concentration, mgg^{-1} or $mgml^{-1}$) of the component under consideration (ascorbic acid in this case), k is the reaction rate constant, and m is the order of the reaction.

Since the degradation was found to follow of first order kinetics, m = 1, integration of eqn (1) over any specified heating period t can be written as

$$\ln([C]_0/[C]_t) = kt \tag{2}$$

where $[C]_0$ is the initial concentration of ascorbic acid at time 0 and $[C]_t$ is the value after heating time t (min).

The relationship of reaction rate to temperature was quantified by the Arrhenius equation, where

$$k = A_0 \exp(-E_a/RT) \tag{3}$$

where E_a is the activation energy of the degradation reaction (kcal mole⁻¹), R is the universal gas constant (1.98 kcal mole⁻¹ K⁻¹), T is absolute temperature (K), and A_0 is a pre-exponential constant (min⁻¹).

RESULTS AND DISCUSSION Concentration of ascorbic acid in drumstick

leaves after the heat treatments

The initial concentration of ascorbic acid in drumstick leaves was 2 g kg^{-1} of leaves. Tables 1 and 2 show the effect of heat treatments on concentration of ascorbic acid in drumstick leaves and in pure solutions of ascorbic acid for different treatment times. It can be seen that the degradation is more or less similar both in drumstick leaves and in pure ascorbic acid solutions up to a temperature of 80 °C. Beyond 80 °C, ascorbic acid was more stable in drumstick leaves, indicating the protective effect of other drumstick constituents. Which protective phytochemicals in drumstick leaves were responsible is not known, and merits further investigation.

Kinetic data for degradation of ascorbic acid

Using linear regression, the degradation data were analyzed using the standard integrated rate equation to determine the overall order and rate constant for the degradation reaction. A correlation coefficient of >0.9 in all the cases confirmed that the degradation of ascorbic acid in pure solution and in leaves and by all the cooking methods studied follows firstorder kinetics at all the temperatures. Figures 1, 2

Table 1. Effect of temperature on vitamin C concentration (mg 100 g⁻¹) of drumstick leaves at various temperatures^{a,b}

Time (min)		Temperature (°C)						
	50	60	70	80	90	100	120	
2	_	_	_	_	_	_	90.76 ± 14	
5	_	_	_	139.5 ± 11	122.5 ± 7	113.1 ± 5	40.78 ± 17	
10	177 ± 8	156 ± 10	132.7 ± 6	109.8 ± 5	106.5 ± 9	97.3 ± 6	21.08 ± 8	
15	_	_	_	_	81.3 ± 4	62.7 ± 3	_	
20	140 ± 4	131 ± 3	116.5 ± 4	79.7 ± 3	58.5 ± 10	40.78 ± 5	8.1 ± 8	
30	119 ± 5	110 ± 6	97.4 ± 5	44.6 ± 8	32.08 ± 6	20.9 ± 11	_	
40	88.6 ± 9	84.6 ± 4	50.3 ± 3	30.5 ± 5	16.9 ± 5	15.15 ± 9	_	
50	60 ± 4	48.3 ± 5	38.8 ± 6	18.7 ± 3	_	_	_	
60	38 ± 4	24.7 ± 8	20.1 ± 8	_	_	_	_	

^a Values are mean \pm SD of three of more individual determinations.

 b The vitamin C content of the drumstick leaves chosen in the study was 200 \pm 6 mg 100 g $^{-1}.$

Table 2. Effect of temperature on vitamin C content (mg $100 g^{-1}$)) of synthetic vitamin C solution at various temperatures ^{a,b}
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	Temperature (°C)						
Time (min)	50	60	70	80	90	100	120
2	_	_	_	_	_	_	81.31±9
5	_	_	_	134.0 ± 10	120.1 ± 6	109.8 ± 4	33.39 ± 18
10	170.4 ± 10	168.7 ± 4	132.7 ± 9	104.4 ± 7	98.3 ± 4	89.87 ± 3	17.2 ± 11
15	_	_	_	_	74.3 ± 12	60.23 ± 7	_
20	146.6 ± 14	135.4 ± 9	116.5 ± 7	84.6 ± 3	48.3 ± 6	33.1 ± 4	7.6 ± 5
30	121.3 ± 3	120.1 ± 5	97.4 ± 5	42.45 ± 6	25.74 ± 2	17.25 ± 8	_
40	096.3 ± 6	84.6 ± 8	50.3 ± 3	31.44 ± 5	16.4 ± 5	10.01 ± 3	_
50	70.00 ± 6	60.2 ± 12	38.8 ± 6	18.7 ± 4	_	_	
60	42.00 ± 5	33.1 ± 3	20.05 ± 8	_	_	_	

^a Values are mean \pm SD of three of more individual determinations.

^b The vitamin C content of the pure solution chosen in the study was 200 mg 100 ml⁻¹.



Figure 1. First-order plot of ascorbic acid degradation in drumstick leaves (\blacklozenge) and in pure solution (\blacktriangle) at 80 °C.



Figure 2. First-order plot of ascorbic acid degradation in drumstick leaves (\blacklozenge) and in pure solution (\blacktriangle) at 90 °C.



Figure 3. First-order plot of ascorbic acid degradation in drumstick leaves (\blacklozenge) and in pure solution (\blacktriangle) at 100 °C.

and 3 show representative plots for drumstick leaves and pure solutions of ascorbic acid at 80, 90 and 100 °C, respectively. The time required for ascorbic acid to degrade to 50% of its original value, $T_{1/2}$, was calculated from the rate constant as 0.693/k. Previous studies also indicated that ascorbic acid degradation follows first-order kinetics in aqueous solutions¹⁴ over a pH range 3.52–7.22,^{15,16} and also for tomato juice,⁷ in a dehydrated food system at pH 6.8⁸ and in orange juice.¹²

Table 3 gives the rate constants and half-life for ascorbic acid in drumstick leaves, and in pure solutions. The rate constants for ascorbic acid degradation in drumstick leaves increased from 0.03 min^{-1} for $50 \,^{\circ}\text{C}$ to 0.12 min^{-1} for $120 \,^{\circ}\text{C}$, and the half-life period decreased from 23.1 min to

5.77 min as the temperature increased from 50 to $120 \,^{\circ}\text{C}$. A similar trend was observed with pure vitamin solutions.

Rate constants reported for ascorbic acid in orange juice¹² ranged from 0.00105 to 0.03471 min⁻¹ for a temperature range of 70–98 °C at °Brix varying from 12.7 to 80.6. The rate constant for ascorbic acid degradation during thermal processing of canned peas at 110 °C has been reported as 0.0005 min⁻¹. The rate constants obtained for ascorbic acid in the present study are higher than the previously reported rate constants. Ascorbic acid is stable in acidic conditions, and hence less degradation and lower rate constants are seen in orange juices. In drumstick leaves, the losses could be higher due to the excessive leaching of ascorbic acid into the water.

Activation energies, E_a (kcal M⁻¹), were calculated as a product of gas constant, $R(1.987 \operatorname{cal} M^{-1} K^{-1})$, and the slope of the graph obtained by plotting $\ln k$ versus 1/T. Figure 4 shows the Arrhenius plot for the degradation of ascorbic acid in drumstick leaves and in pure ascorbic acid solutions. The activation energies in the present study were $4.39 \text{ kcal mol}^{-1}$ for drumstick leaves and $5.37 \text{ kcal mol}^{-1}$ for pure vitamin solution. Studies have reported wide variation in the activation energy for ascorbic acid degradation in different food systems. The activation energy for ascorbic acid in canned peas has been reported as 41 kcal mol⁻¹.¹⁷ The activation energy for aerobic oxidation at pH 5.6 and in the temperature range $60-85 \degree C$ was 18 kcal mol^{-1} .¹⁶. The $E_{\rm a}$ was evaluated as $18.3 \,\rm kcal \, mol^{-1}$ for ascorbic acid degradation in a dehydrated model system at water activity of 0.65.⁸ Lee *et al*⁷ investigated the degradation of ascorbic acid during storage of canned tomato juice and the E_a was 3.3 kcal mol⁻¹. The E_a for orange juice and orange serum for a temperature



Figure 4. Arrhenius plot for thiamine degradation in drumstick leaves (\blacklozenge) and in pure solutions (\blacktriangle) .

Table 3. Rate constant (k min⁻¹) and half-life (min) of vitamin C degradation in drumstick leaves and in pure system at pH 6.5

	Drumstick	leaves	Synthetic vitamin C (pH 6.5)		
Temperature (°C)	Rate constant $k(\min^{-1})$	$t_{1\setminus 2}$ (0.693/k, min)	Rate constant k (min ⁻¹)	$t_{1\setminus 2}$ (0.693/k, min)	
50	0.030	23.1	0.027	25.60	
60	0.036	19.25	0.031	22.35	
70	0.038	18.24	0.036	19.25	
80	0.045	15.40	0.045	15.40	
90	0.058	11.94	0.060	11.50	
100	0.060	11.50	0.080	8.66	
120	0.120	5.77	0.125	5.54	

The standard errors in *k* values were less than 6×10^{-4} .

range of 70-98 °C and °Brix 12.6-80.6 was in the range 27.5-30 kcal mol⁻¹.

The difference in activation energies for ascorbic acid in the present study for drumstick leaves and for the pure vitamin solution from the reported values may be due to the different temperature ranges used in the studies and to different environmental conditions, such as oxygen, moisture, pH and compositions of food products.

Time-temperature data of the three modes of cooking

To extend the results obtained from the isothermal heating experiments to the non-isothermal ones encountered in the three modes of cooking, viz openpan cooking, pressure cooking and cooking in the eco cooker, time-temperature data during the processing of each was recorded (Fig 5).

Degradation profiles of ascorbic acid under the three modes of cooking

Ascorbic acid degradation was followed in each of these modes of cooking for drumstick leaves using the data for isothermal conditions. From Table 4 it can



Figure 5. Time-temperature profiles of the different cooking methods used.

Table 4. Degradation profile and kinetics of vitamin C in drumstick
leaves at different cooking methods ^a

Method of cooking	Time (min)	Vitamin C concentration (mg 100 g^{-1})	Rate constant k^{b} (min ⁻¹) (r^{2})	t _{1\2} (0.693/ k, min)
Open-pan cooking	5	175 ± 05		
-	10	135 ± 04	0.041 (0.99)	17.00
	20	076 ± 06		
Pressure- cooking	5	168 ± 08		
	10	120 ± 10	0.100 (0.95)	06.93
	15	061 ± 15		
Eco cooking ^c	10	184 ± 13		
	20	128 ± 17	0.050 (0.93)	14.00
	30	066 ± 09		

^a Results are mean SD of three determinations.

^b Calculated from semi-log plot of ln C_0/C_t versus t.

 $^{\rm c}$ Held for 30 min as per the protocol recommended for cooking with the Eco cooker.

be seen that the degradation was of a similar order of magnitude in all the modes of cooking.

Prediction of ascorbic acid loss during non-isothermal heating process

To predict the amount of ascorbic acid degradation occurring in drumstick leaves during a given nonisothermal heating process, the following equation was derived from the integrated first-order rate law:

$$k_i = A_0 \exp(-E_a/RT_i) \tag{4}$$

Where k_i is the rate constant at time t_i . E_a is the activation energy of the reaction, R is the gas constant, T is absolute temperature and A_0 is a pre-exponential constant, which were previously calculated for the isothermal heating process. The rate constant k_i at each temperature was calculated using eqn (4) and, substituting for T from the time-temperature data of the non-isothermal heating process. Knowing the rate constant k_i , the rate dC/dt_i , the amount degraded during the time interval zero to t_i and the final concentration C can be calculated as follows:

Rate = rate constant $k_i \times \text{initial concentration } C$ Amount degraded during $t_i(\Delta C) = \text{rate} \times t_i$ Concentration after time $t_i = C - \Delta C$

These calculations were continued for the entire time period (heating and constant temperatures) at which each cooking process was conducted. An MS Excel-based computer program was used to calculate the above parameters.

The total amount degraded after complete cooking = $\sum \Delta C$. The final concentration thus will be = $C_0 - \sum \Delta C$, where C_0 is the initial concentration of ascorbic acid. The resulting predictions and the actual degradation obtained experimentally are given in Table 5. A reasonable agreement between the actual and the predicted degradation/retention of ascorbic acid was obtained. Using this method, the degradation of ascorbic acid can be predicted for any processing method, if the time-temperature profile of that processing operation is known. Accuracy of the prediction can be improved by reducing the time interval of the constant temperature assumption.

CONCLUSIONS

Slow cookers, as exemplified by an eco cooker, are fuel efficient and show similar magnitude of

 Table 5. The actual and predicted retention of ascorbic acid in the cooking methods

	Drumstick leaves (mg 100 g^{-1})			
Cooking method	Actual retention	Predicted retention		
Open pan	76	56		
Pressure	61	66		
Eco	66	45		

retention of ascorbic acid as open-pan cooking and pressure-cooking. The need to study the other watersoluble vitamins is also warranted. On the basis of the ascorbic acid retention, and fuel savings, an overall judgment in favour of the slow eco cooker is suggested.

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