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ARTICLE

Influence of Simultaneous Variations in Temperature and Relative Humidity on Chemical Stability of Two Vitamin C Forms and Implications for Shelf Life Models

Ashley N. Hiatt,[†] Lynne S. Taylor,[‡] and Lisa J. Mauer*,[†]

[†]Department of Food Science, Purdue University, 745 Agriculture Mall Drive, West Lafayette, Indiana 47907, and [‡]Department of Industrial and Physical Pharmacy, Purdue University, 575 Stadium Mall Drive, West Lafayette, Indiana 47907

Vitamin C powder ingredients are popular food fortificants and are among the most commonly added nutrients. While information on degradation kinetics and shelf life of vitamin C exists, current models are limited in their applicability to systems where relative humidity (RH) and temperature are varied simultaneously, and where phase transformations occur. This study monitored stability of two forms of vitamin C (sodium ascorbate and ascorbic acid) under various RH and temperature conditions. Vitamin C was measured colorimetrically, and stability data were used to improve understanding of vitamin C shelf life when deliquescence occurs during storage. RH and temperature both significantly impacted vitamin stability, though RH had a larger effect. Vitamin dissolution preceded extensive degradation and was found to be a good predictor of vitamin C loss. This research highlights the importance of considering phase transformations when constructing shelf life models and maintaining vitamin C in the solid state for enhanced stability.

KEYWORDS: Vitamin C; shelf life; temperature; relative humidity; stability

INTRODUCTION

The fortification of powder products and supplements with vitamins and other bioactive compounds is becoming increasingly prevalent. Vitamin C is a popular ingredient for food fortification, and is one of the most commonly added nutrients (*1*). Additionally, vitamin premixes used for food product fortification are typically supplied in powder form and are subject to strict labeling regulations. Therefore, greater understanding of vitamin C degradation kinetics during exposure to a variety of temperature and relative humidity (RH) conditions is of value.

Since vitamin C is highly unstable and its content in foods must be declared on nutrient labels, it is commonly monitored to determine shelf life. Vitamin C is also frequently used as an index of the overall nutrient quality of foods during processing and storage because of its sensitivity to processing conditions; hence it is important to predict its retention. Additionally, losses of vitamin C are related to deteriorative reactions in foods beyond loss of nutritional quality, such as development of undesirable color and flavor changes (*2*, *3*). Common shelf life estimates are obtained by exposing a food product to an abuse condition for selected storage times and evaluating loss of a specified quality attribute. Monitoring deterioration of vitamin C until it no longer meets its declared label value is one way to determine the product's shelf life (*4*). While temperature is frequently the accelerating factor chosen, water activity (a_w) is the next most important factor influencing degradation rate (*5*, *6*). Accelerated shelf life testing (ASLT) is an approach used by the food industry to set product shelf life dates based on minimum acceptable quality. However, this approach limits the usefulness of shelf life data due to changes in reaction mechanism or use of only a single accelerating factor, such as temperature, during testing (*7*).

An increase in moisture content within a powder system has been shown to enhance degradation rates of sensitive ingredients (*8*). Additionally, a_w is temperature dependent, capable of increasing as temperature increases for many foods, or decreasing if temperature increases result in further dissolution of solutes. This can have serious implications for storage stability when temperature is not constant, allowing a_w to potentially increase sufficiently to allow spoilage or deterioration, or altering food properties and stability (*8*). Water activity can impact kinetic parameters; however, in many shelf life studies a_w is not taken into account or is simply controlled at one water activity level. These factors can interact in a sometimes unpredictable way; therefore, potential interactions must be investigated. Currently, however, synergistic effects of changing relative humidity (RH) and temperature on deterioration rates are poorly understood.

Attempts to determine the effect of RH and temperature on the degradation rate in a food system typically control one factor at a set level while varying the second. Reaction rates for vitamin C degradation in infant formula and a model system were correlated with water activity, increasing as a_w increased (*9*, *10*). A separate study determined that reaction rates for ascorbic acid degradation during storage at constant temperature were linearly related to a_w (*11*). Few studies vary RH and temperature simultaneously, resulting in limited kinetic data on vitamin C loss during combined temperature−RH treatments. Additionally, studies that have attempted to elucidate the impact of RH on ingredient

*To whom correspondence should be addressed. Tel: (765) 494-9111. Fax: (765) 494-7953. E-mail: mauer@purdue.edu.

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degradation, either individually or in combination with temperature, typically fail to investigate the impact of the mechanism by which RH is affecting stability. Glass transition models for kinetic studies combine the effect of both temperature and moisture content, but may only be useful if the reaction is diffusion limited and would not be appropriate for crystalline materials (*4*). Since shelf life analysis depends on knowledge of the deterioration mechanism, phase transitions that occur during testing could largely impact shelf life predictions (*12*). Sufficient data on the specific role of variable RH in degradation of sensitive ingredients in powder systems, independent of temperature, are lacking. Therefore, more work is necessary to determine how phase transitions impacted by RH, such as deliquescence, are affecting the stability of vitamin C.

Using multiple accelerating factors, such as temperature and RH, allows for improved shelf life predictions and models. Current shelf life models could be enhanced by establishing the effect of RH and temperature, separately and synergistically, on degradation of vitamin C in powder formulations. Investigating the impact of these two parameters in conjunction with each other would further the understanding of their complex interrelationship with vitamin degradation reactions in a dynamic system. The objective of this study was to enhance understanding of the relationship between deliquescence and shelf life for two common forms of vitamin C, ascorbic acid and sodium ascorbate (which have different deliquescence points), when exposed to various temperature and RH conditions.

MATERIALS AND METHODS

Materials. Sodium ascorbate was purchased from Sigma Aldrich Co. (St. Louis, MO), and ascorbic acid was obtained from Mallinckrodt-Baker (Phillipsburg, NJ). Materials for preparing reagents for the microplate reader assay included orthophosphoric acid (Alfa Aesar, Ward Hill, MA), trichloroacetic acid (TCA) and 2,2-bipyridine (Mallinckrodt-Baker, Phillipsburg, NJ), iron chloride (EMD Chemicals, Gibbstown, NJ), ethanol (Aaper Alcohol & Chemical Co., Inc., Shelbyville, KY), and potassium phosphate monobasic and dibasic (Mallinckrodt-Baker, Inc., Paris, KY). Salts used to create saturated salt solutions and control RH in environmental chambers included indicating Drierite (trademark of W. A. Hammond Drierite Co., Ltd., Xenia, OH), $Mg(NO_3)_2$, $CoCl_2$, NaCl (Sigma-Aldrich, Inc., St. Louis, MO), KCl, and K_2SO_4 (Mallinckrodt-Baker, Phillipsburg, NJ).

Controlled RH and Temperature Storage. Samples were stored in desiccators above the following saturated salt solutions to give known RHs: magnesium nitrate (54%); cobalt chloride (64%); sodium chloride (75%); potassium chloride (85%), and potassium sulfate (98%). Indicating Drierite was used to create a 0% RH storage desiccator. For 25 °C storage, desiccators were left at room temperature. For 4 °C storage, desiccators were placed in a controlled temperature storage room. For 35 and 40 °C storage, desiccators were placed into water-jacketed incubators (Forma Scientific, Inc., Marietta, OH). The RH of each chamber was verified by digital hygrometer (traceable humidity/temperature/dew point meter, Control Co., Friendswood, TX) or water activity (AquaLab 3TE, Decagon Devices, Inc., Pullman, WA), and variation from known RH was ±3%.

Microplate Reader Assay. A colorimetric microplate reader assay was performed to measure amounts of reduced ascorbate remaining in samples after treatment. Samples were removed from storage, weighed, and diluted in distilled H_2O. A subsequent dilution step was achieved in 6% TCA. The microplate reader method of Stevens et al. (*18*) for reduced ascorbate was followed, slightly modified by analyzing samples on an AD/LD 340 Absorbance Detector microplate reader (Beckman Coulter, Inc., Fullerton, CA) at 570 nm. Ascorbate content remaining after storage was determined by comparing to standard curves of known concentrations of either ascorbic acid or sodium ascorbate from 0.25 nmol/μL to 1.5 nmol/μL.

Moisture Sorption Analysis. Gravimetric sorption analysis was performed using a symmetrical gravimetric analyzer (SGA-100) (VTI Corporation, Hialeah, FL) at 25 and 40 °C in order to determine the critical relative humidity (RH_0) of the deliquescent solids sodium ascorbate and ascorbic acid (*19*). A sample weight of 10−15 mg for each vitamin C form was used. Prior to sorption analysis, samples were dried at 60 °C in the sorption analyzer. The settings for the sorption analyzer were as follows: equilibrium criterion for the drying step of 0.01% w/w in 2 min, maximum drying time of 30 min, and step equilibrium criterion of 0.001% w/w in 5 min with a maximum step time of 60 min. During the experiment, samples were exposed to increasing RH (from 0 to 94% RH), increasing at 10% intervals from 20 to 70% RH and at 2% intervals from 70 to 94% RH. RH_0 was determined from the point in the isotherm at which the sample began to rapidly sorb moisture (*19*).

Kinetic Study. All samples were prepared and analyzed in triplicate for each time, temperature, and water activity combination. A 200 mg sample of each vitamin C form was stored in 20 mL glass vials at the stated condition. Five time points were selected, and degradation of vitamin C was monitored every two weeks for eight weeks. Samples that achieved at least 50% degradation by the end of the storage period were further analyzed for degradation kinetics.

Shelf Life Plots and Calculation of Q_{10} and Q_A Values. Kinetic plots of both ascorbic acid and sodium ascorbate degradation were used to determine the half-life of each vitamin C form at a given combination of storage RH and temperature. These values were then plotted against either a_w or temperature to obtain shelf life plots. The use of a Q_{10} value for determining shelf life is commonly used in the food industry and is a practical approach for relating loss of a quality parameter, such as vitamin deterioration, to temperature. The Q_{10} of a reaction is the increase in rate for a 10 °C increase in temperature (*6*). A Q_{10} value was calculated using the slope (b) of a plot of ln(half-life) versus temperature from the following equation (*20*):

$$Q_{10} = \exp(10|b|)$$

The use of Q_A for describing degradation in relation to a_w changes can be applied for shelf life understanding. The Q_A value represents the decrease in half-life as a result of increasing a_w by 0.1 unit (*16*, *17*). Q_A values were obtained by plotting the log of half-life versus a_w to obtain the slope, B, and using the following equation (*20*):

$$Q_A = 10^{0.1|B|}$$

Statistical Analysis. All samples were prepared in triplicate. A completely randomized three factor factorial design was used for studying the effects of RH, temperature, and time on the stability of vitamin C. The data were analyzed using ANOVA models. Individual differences were tested using Tukey's multiple means comparison procedure. All statistical analysis procedures were conducted using PC SAS software and $\alpha = 0.05$.

RESULTS

Effect of Temperature on Moisture Sorption and RH_0. Gravimetric moisture sorption analysis was used to measure RH_0 for each vitamin C form at 25 and 40 °C. Ascorbic acid exhibited a higher deliquescence RH than sodium ascorbate at a given temperature (**Figure 1**, **Table 2**). The RH_0 for ascorbic acid at 25 °C was 98% RH, while the RH_0 for sodium ascorbate at 25 °C was 86% RH. Increasing the temperature to 40 °C decreased RH_0 for both forms of vitamin C, with a deliquescence point of 86% RH for ascorbic acid and a RH_0 of 82% RH for sodium ascorbate at the higher temperature.

Effect of Temperature (25 °C vs 40 °C) on Ascorbic Acid and Sodium Ascorbate Stability. In general ascorbic acid was stable to all RH conditions (54−98% RH) at 25 °C for 8 weeks (**Figure 2A**). At 40 °C ascorbic acid was stable at RHs below its RH_0 (75% and 85% RH), but exhibited significant degradation once exposed to 86% RH, greater than RH_0 ($p < 0.0001$) (**Figure 2A**). After 8 weeks of storage at 98% RH and 25 °C, ascorbic acid appeared as slightly yellowed crystals with visible moisture present, while at 98% RH and 40 °C the originally white ascorbic acid crystals had formed a dark brown liquid. Sodium ascorbate was stable to RH conditions below its RH_0 of 86% RH

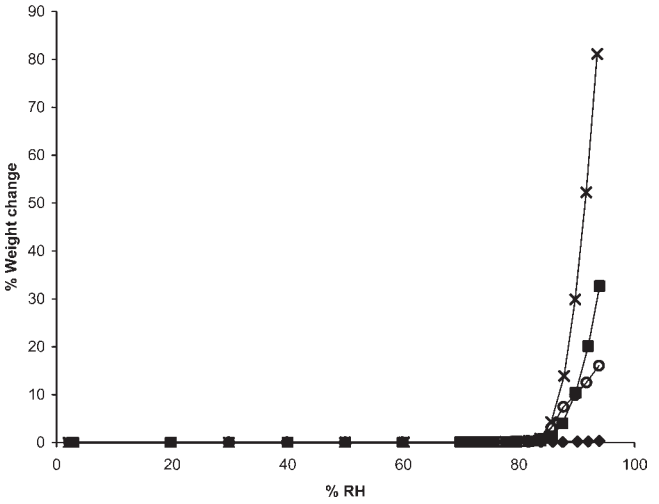

Figure 1. Moisture sorption isotherms of ascorbic acid and sodium ascorbate at 25 or 40 °C. Vitamin forms and temperature: (—◆—) ascorbic acid 25 °C; (—■—) sodium ascorbate 25 °C; (—○—) ascorbic acid 40 °C; (—×—) sodium ascorbate 40 °C.

(54, 64, 75% RH) at 25 °C, but complete degradation was observed after 8 weeks of storage near (85% RH) and above (98% RH) RH_0 (**Figure 2B**). It appears that at room temperature appreciable degradation does not commence until RH_0 is approached or exceeded. At 40 °C degradation began at RH conditions below RH_0: sodium ascorbate was stable at 54% and 64% RH, but degradation occurred during storage at 75%, 85%, and 98% RH (**Figure 2B**). At the higher temperature (40 °C), a greater extent of browning was observed compared to sodium ascorbate stored at 25 °C at all storage RHs.

Effect of RH (0% vs 75% RH) on Ascorbic Acid and Sodium Ascorbate Stability. During storage at 0% RH ascorbic acid was stable at all temperatures studied (4, 25, 35, 40 °C). Similarly, temperature did not appear to influence ascorbic acid stability when stored at 75% RH. Degradation at this RH did not differ significantly between any of the storage temperatures throughout the 8 week storage period ($p = 0.9635$ to $p = 1.000$).

Sodium ascorbate stability was also not affected by storage temperature when samples were kept at dry conditions (0% RH). During 75% RH storage, sodium ascorbate was stable at 4 and 25 °C, but exhibited almost complete degradation after 8 weeks of storage at 35 and 40 °C (**Figure 3**). Enhanced sodium ascorbate degradation occurred at temperatures exceeding room temperature (at 35 and 40 °C) at RHs above and just below RH_0 (75, 85, and 98% RH).

Influence of Both RH and Temperature on Ascorbate Stability. RH significantly affected vitamin C stability ($p < .0001$). The proximity of storage RH to RH_0 affected ascorbic acid and sodium ascorbate stability, with both vitamin C forms exhibiting stability at RH conditions below RH_0 at room temperature (**Figure 2A**,**B**). The same trend is true at elevated temperatures for RHs much less than RH_0 of sodium ascorbate (54 and 64% RH). This suggests that there is a level of moisture uptake beyond which vitamin C is more labile. For those conditions at which ascorbic acid was stable (0% RH and 4, 25, 35, and 40 °C; 75% RH and 4, 25, 35, and 40 °C; 25 °C and 85% and 98% RH; 40 °C and 85% RH), end point weight change (after 8 weeks of storage), indicative of moisture uptake, ranged from a loss of 1.1 ± 0.01% w/w to a gain of 41.7 ± 7.8% w/w. This corresponds to a loss of 0.16 ± 0.2 mmol of water and to a gain of 4.7 ± 0.9 mmol of water per mmol of solid ascorbic acid. At 98% RH and 40 °C, where almost complete degradation of ascorbic acid

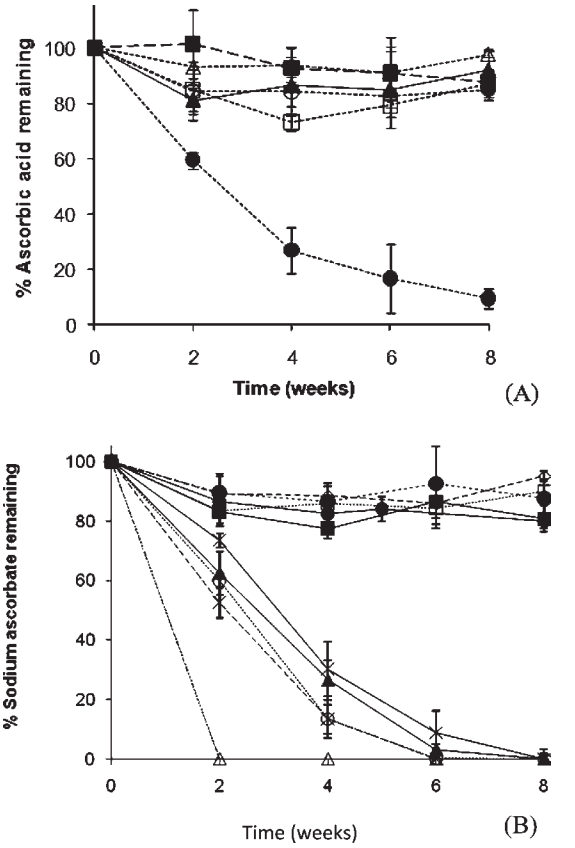

Figure 2. Stability of each vitamin C form during storage up to 8 weeks at 25 or 40 °C and various storage RH. Bars show standard errors for each sample. (**A**) Ascorbic acid stability during storage at 75%, 85%, or 98% RH. Storage RH: (- - -△- - -) 75% 25 °C; (- - -□- - -) 85% 25 °C; (- - -○- - -) 98% 25 °C; (—▲—) 75% 40 °C; (- - -■- - -) 85% 40 °C; (—●—) 98% 40 °C. (**B**) Sodium ascorbate stability during storage at 54%, 64%, 75%, 85%, or 98% RH. Storage RH: (—●—) 54% 25 °C; (—■—) 64% 25 °C; (— —●— —) 75% 25 °C; (—×—) 85% 25 °C; (—▲—) 98% 25 °C; (— —◇— —) 54% 40 °C; (- - -□- - -) 64% 40 °C; (- - -○- - -) 75% 40 °C; (— —×— —) 85% 40 °C; (- - -△- - -) 98% 40 °C.

occurred, end point weight gain equaled 80.6 ± 5.8%, or 9.1 ± 0.6 mmol of water per mmol of solid. However, at this same storage RH (98%) but lower temperature (25 °C), only 15.0 ± 3.6% degradation occurred when 4.7 ± 0.9 mmol of water was present. It appears that moisture uptake in excess of ~5 mmol of water for every ~1 mmol of ascorbic acid results in reduced stability.

For sodium ascorbate, end point moisture uptake at conditions where stability was observed (0% RH and 4, 25, 35, and 40 °C; 75% RH and 4 and 25 °C; 25 °C and 54% and 64% RH; 40 °C and 54% and 64% RH) ranged from -0.3 ± 0.3% w/w to 1.0 ± 0.2% w/w, or equivalently a loss of 0.04 ± 0.03 mmol water to a gain of 0.11 ± 0.02 mmol water per mmol of ascorbate. At conditions where sodium ascorbate was chemically labile (75% RH and 35 and 40 °C; 25 °C and 85% and 98% RH; 40 °C and 85% and 98% RH), end point moisture uptake ranged from 14.8 ± 0.4% w/w (1.6 ± 0.03 mmol water) to 278.9 ± 5.0% w/w (31.0 ± 0.5 mmol water). For all sodium ascorbate samples where moisture uptake exceeded the molar amount of solid present (~1 mmol), appreciable degradation was observed.

Based on a comparison of the mean square errors from the ANOVA results, RH had the largest mean square error value and thus the largest effect on vitamin C stability. Additionally, since both vitamin C forms were stable at all temperatures studied

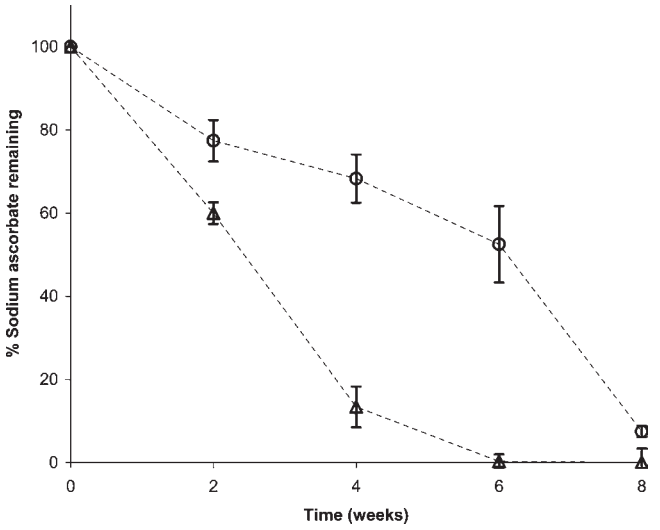

Figure 3. Stability of sodium ascorbate during storage up to 8 weeks at 35 and 40 °C and 75% RH. Storage temperature: (---○---) 35 °C 75%; (---△---) 40 °C 75%.

when stored in dry conditions (0% RH) and ascorbic acid was stable at all temperatures at 75% RH (below its RH_0), RH appears to have a greater impact on stability than temperature. Sodium ascorbate was also stable at 40 °C for RHs well below its RH_0 of 82% (54 and 64% RH) (**Figure 2B**); however, at the higher RH storage conditions close to or exceeding RH_0 for sodium ascorbate (75%, 85%, 98% RH), degradation was observed at the elevated temperatures of 35 and 40 °C (**Figure 3**).

Relationship between Moisture Uptake and Vitamin C Stability. Both ascorbic acid and sodium ascorbate exhibited minimal weight gain during storage at 0% and 75% RH at all temperatures studied (4, 25, 35, 40 °C) (data not shown). At higher RH conditions (85% and 98% RH) both vitamin C forms demonstrated the greatest weight gain attributable to moisture uptake at 98% RH. Ascorbic acid did not show appreciable moisture uptake at either 25 or 40 °C at RHs below its deliquescence RH (98% RH) (**Figure 4A**). Sodium ascorbate moisture uptake was generally greater at 85% RH at 25 and 40 °C than at lower RHs (54%, 64%, 75% RH) ($p < 0.0001$). Sodium ascorbate end point weight gain following storage at 40 °C was less than that observed during storage at 25 °C at 85% and 98% RH (**Figure 4B**).

For samples exhibiting at least 50% vitamin degradation by the end of the 8 week storage period, a correlation between weight gain and vitamin loss was observed. Sodium ascorbate stored under the following conditions demonstrated a trend of decreased stability with increasing weight gain: 75% and 35 °C; 75% RH and 40 °C; 85% RH and 25 °C; 85% RH and 40 °C; 98% RH and 25 °C; 98% and 40 °C (**Figure 5A**,**B**). No ascorbate sample at storage conditions at which less than 50% degradation occurred exceeded a moisture uptake of 1.0 ± 0.2% (0.11 mmol water per mmol solid). For samples where instability was observed, weight gain was at least 9.9 ± 1.8% (1.1 mmol of water per mmol of solid). Therefore, as sample weight gain approaches 1 mmol of water for each 1 mmol of solid present, sodium ascorbate becomes more chemically labile.

Plotting the theoretical percent vitamin C dissolved versus percent vitamin C remaining for each vitamin form further highlights the relationship between moisture sorption and vitamin stability. Both vitamin C forms were generally stable under storage conditions where minimal vitamin dissolution occurred (**Figure 6A**). Once approximately 10% of the solid sodium ascorbate had dissolved, stability declined (**Figure 6B**).

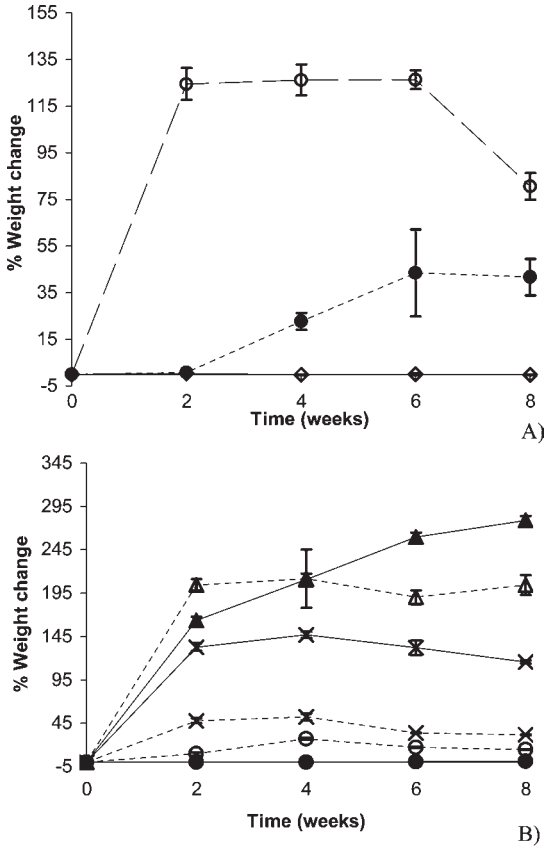

Figure 4. % Weight change for each vitamin C form stored at different RH conditions and at 25 or 40 °C for up to 8 weeks. Error bars show standard errors for that sample. (**A**) Ascorbic acid samples stored at different RH conditions at 98% RH and 25 and 40 °C and 75% RH at 25 °C. Weight gain trends for ascorbic acid stored at 25 and 40 °C and below 98% RH were similar to that observed at 75% RH and 25 °C and are not shown. Storage RH: (—◆—) 75% RH 25 °C; (---●---) 98% RH 25 °C; (— —○— —) 98% RH 40 °C. (**B**) Sodium ascorbate samples stored at different RH conditions at 25 or 40 °C. Weight gain trends for samples stored below 75% RH at both temperatures were similar to that shown for 75%RH and 25 °C and are not shown. Storage RH: (—●—) 75% 25 °C; (—×—) 85% 25 °C; (—▲—) 98% 25 °C; (---○---) 75% 40 °C; (---×---) 85% 40 °C; (---△---) 98% 40 °C.

Similarly, for ascorbic acid it appears that stability was decreased once approximately 40% of the vitamin had gone into solution.

Degradation Kinetics. A good fit to first-order kinetic plots was obtained for vitamin C samples that achieved at least 50% degradation during the storage period. R^2 values ranged from 0.72 to 0.99 for these conditions (**Table 1**). Generally, rate constants for first-order reactions were of the same magnitude for sodium ascorbate, ranging from 0.08 to 0.30 day^{-1}. The rate constant tended to increase with increasing RH, as well as with temperature (**Table 1**). The highest k value was observed at 98% RH and 40 °C. A 50% degradation of ascorbic acid within the experimental conditions was only achieved at 98% RH and 40 °C. The k value for this reaction was 0.043 day^{-1} (**Table 1**).

Rate constants for loss of vitamin C in samples that did not exhibit degradation in excess of 50% of the starting amount generally had poor R^2 values for all simple kinetic order plots. Rate constants were determined by plotting vitamin loss over time as concentration of vitamin C remaining, $\ln(A/A_0)$, and $1/A_0$ (where A equals the concentration of vitamin C remaining at the end of the storage period and A_0 is the initial vitamin C concentration); the reaction order plot with the best fit (R^2 closest

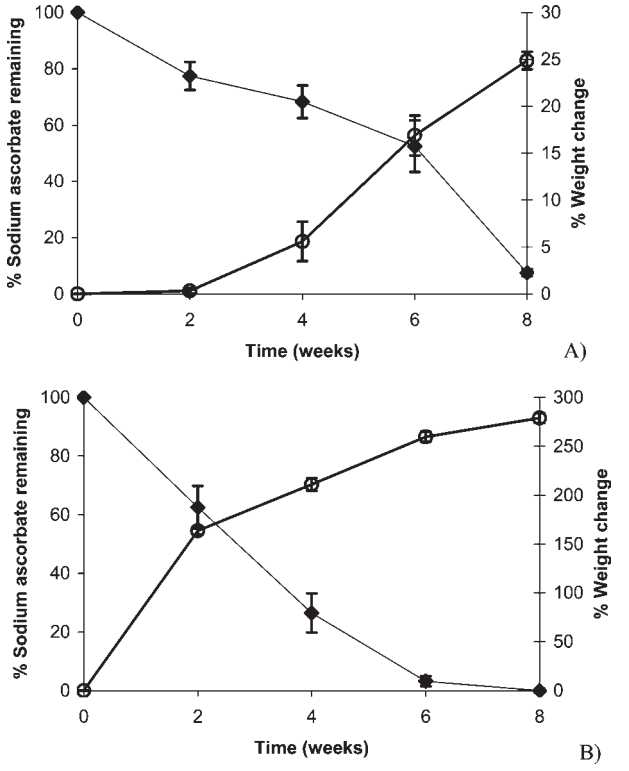

Figure 5. Representative graphs demonstrating a relationship between % weight change and sodium ascorbate degradation during storage at select RH and temperature conditions up to 8 weeks. % sodium ascorbate remaining and % weight change in each chart: (◆) % remaining; (○) weight change. (**A**) % sodium ascorbate remaining and % weight change over time during storage at 35 °C and 75% RH. (**B**) % sodium ascorbate remaining and % weight change over time during storage at 25 °C and 98% RH.

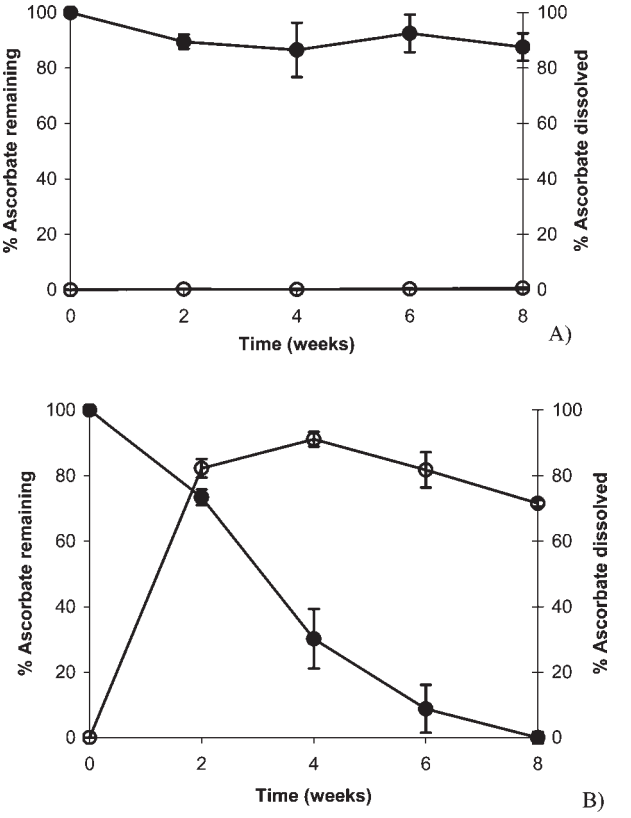

Figure 6. Representative graphs demonstrating a relationship between % vitamin C dissolved and vitamin C degradation at select RH and 25 °C up to 8 weeks. Data for sodium ascorbate is shown, and trends for ascorbic acid were similar. % sodium ascorbate dissolved and % sodium ascorbate remaining in each chart: (●) % remaining; (○) % dissolved. (**A**) % sodium ascorbate remaining and % dissolved over time during storage at 75% RH and 25 °C. (**B**) % sodium ascorbate remaining and % dissolved over time during storage at 85% RH and 25 °C.

to 1) was selected. At lower RHs where minimal degradation occurred, R^2 values were typically very similar for both zero- and first-order reaction plots. Sodium ascorbate degradation at 75% RH and 4 °C best fit a zero-order kinetic plot unlike the rest of the samples; however, the poor fit of the data to either model reduces the credibility of this result.

Shelf Life Plots. The shelf life of ascorbic acid related to temperature at 75% RH is shown in **Figure 7**. Both vitamin C forms were temperature sensitive, with a decreased half-life as temperature increased, though sodium ascorbate was less stable at all storage temperatures than ascorbic acid. Q_{10} values for sodium ascorbate and ascorbic acid at 75% RH were 1.8 and 1.2, respectively. Similarly, vitamin C half-life decreased as a_w increased. A representative Q_A plot for sodium ascorbate is shown in **Figure 8**. Again, sodium ascorbate was less stable than ascorbic acid at all storage RHs. Q_A values for sodium ascorbate at 25 and 40 °C were 1.9 and 3.4, respectively, indicating the combined influence of a_w and temperature on vitamin stability. This suggests that the rate of sodium ascorbate degradation increases 90% for every 0.1 unit increase in a_w at 25 °C. Q_A values were also enhanced for ascorbic acid as temperature increased from 25 °C (Q_A = 1.1) to 40 °C (Q_A = 2.8). Comparing Q_A values for sodium ascorbate and ascorbic acid indicates that ascorbic acid is less influenced by increases in a_w than sodium ascorbate.

DISCUSSION

Impact of Temperature on Vitamin C RH_0. Ascorbic acid and sodium ascorbate deliquescence RHs were found to be 98% RH and 86% RH at 25 °C, in accordance with previously reported values (*21*, *22*). RH_0 decreased with increasing temperature as a result of enhanced water solubility of the vitamin C forms at higher temperatures (**Table 2**). Solubility enhancement, and thus the reduction in RH_0, was greater for ascorbic acid when temperature was increased from 25 to 40 °C than that observed for sodium ascorbate. This is the first report of the effects of temperature on the deliquescence points of vitamin C ingredients.

Table 1. Reaction Order and R^2 for Degradation Kinetics of Two Vitamin C Forms (Sodium Ascorbate and Ascorbic Acid) during Storage at RH and Temperature Conditions up to 8 Weeks That Resulted in at Least 50% Degradation[a]

vitamin C form	storage RH (%)	storage temp (°C)	reaction order[b]	k (day^{-1})	R^2
sodium ascorbate	75	35	first	0.015	0.92
sodium ascorbate	75	40	first	0.10	0.96
sodium ascorbate	85	25	first	0.081	0.91
sodium ascorbate	98	25	first	0.087	0.95
sodium ascorbate	85	40	first	0.094	0.93
sodium ascorbate	98	40	first	0.302	0.99
ascorbic acid	98	40	first	0.043	0.99

[a] R^2 values for samples not shown were less than 0.8. [b] Reaction order was determined by plotting concentration of vitamin C remaining or $\ln(A/A_o)$ versus time and choosing which plot best fit the data as determined by the R^2 value. When similar R^2 values were obtained for both zero and first order plots, first order was selected based on previous reports of first-order degradation kinetics for vitamin C.

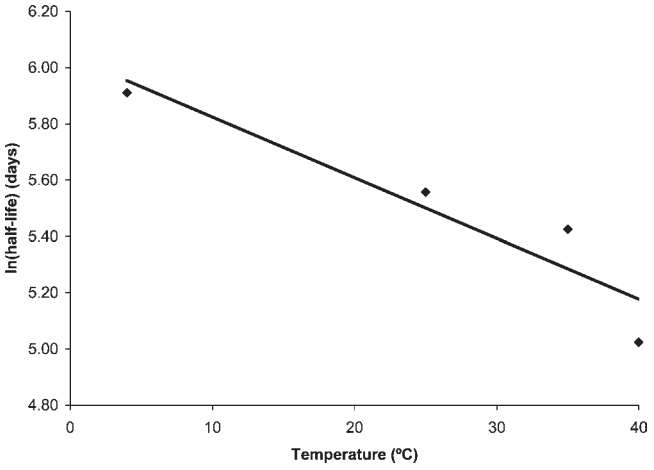

Figure 7. Shelf life plot for ascorbic acid at constant RH of 75% with increasing storage temperature ranging from 4 to 40 °C.

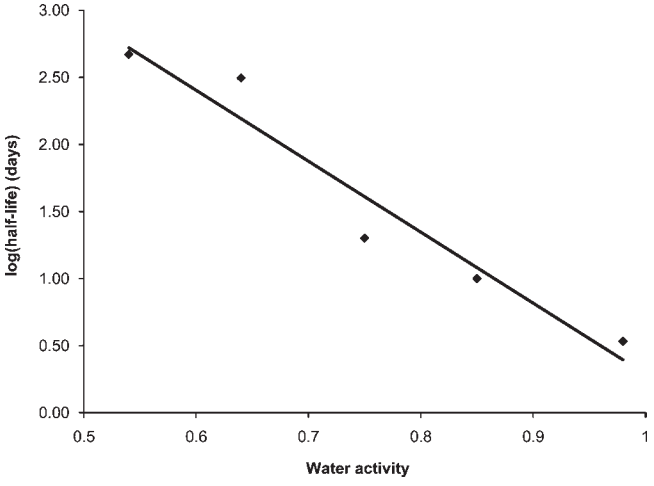

Figure 8. Q_A plot for sodium ascorbate stored at 40 °C and a_w's ranging from 0.54 to 0.98.

Table 2. Solubility and RH_0 Values Measured Experimentally and Obtained from the Literature for Both Sodium Ascorbate and Ascorbic Acid at 25°C and 40°C

	25 °C		40 °C	
vitamin C form	solubility (mol/kg)	RH_0	solubility (mol/kg)	RH_0
sodium ascorbate	3.13[a]	86%	3.37[c]	82%
ascorbic acid	1.89[a]	98%	2.95[b]	86%

[a] Referenced from ref *23*. [b] Referenced from ref *24*. [c] Estimated from reported values of sodium ascorbate solubility at 25 and 75 °C, referenced from ref *23*.

Impact of RH and Temperature on Vitamin C Stability. Both RH and temperature conditions influenced stability of ascorbic acid and sodium ascorbate. RH had the largest impact on vitamin C degradation, and a synergistic effect between RH and temperature occurred that altered degradation. Generally, sodium ascorbate was stable at RHs well below RH_0, even at elevated storage temperatures, while at and above RH_0, severe degradation occurred at all temperatures. A similar trend was observed with ascorbic acid. Vitamin C solubility is known to increase as temperature increases (*23*, *24*); therefore, the increase in degradation at higher storage temperatures is likely due to enhanced dissolution of the vitamin at these conditions as evidenced by the increased moisture sorption observed at the higher temperatures. Additionally, saturated solutions of the two vitamin C forms differ in pH, with values of ~2 and ~5.6 for ascorbic acid and sodium ascorbate, respectively. Since vitamin C is more susceptible to degradation at higher pH, this difference could provide another source of destabilization for sodium ascorbate compared to ascorbic acid.

Dependence of the rate of vitamin C degradation on temperature has been shown to deviate from the Arrhenius relationship when phase changes are involved (*6*, *12*). These systems have continually changing amounts of dry and dissolved vitamin, which may alter the susceptibility of vitamin C to degradative reactions (*4*). At low moisture contents vitamin C is essentially in the solid state and can be expected to follow solid state degradation reactions (*25*), which are thought to differ from those in solution (*26*). Generally, solution state ascorbic acid degradation has been described as the result of oxidation to dehydroascorbic acid followed by subsequent hydrolysis to further end products (*2*, *27*, *28*). Evidence of differences in degradation was provided by Shephard et al. (*29*) with the finding that, unlike in the solution state, furans were not detected during solid phase ascorbic acid degradation. Production of different degradation products could result in alterations to vitamin C stability, depending on the solubility (and thus contribution to deliquescence lowering) of the end products and their impact on the solution pH (*13*, *15*, *29*). Water content and a_w are known to influence reaction rates (*30*), thus the introduction of moisture into the dry vitamin C system over time would alter degradation kinetics. Increasing a_w can either increase or decrease reaction rates, depending on the influence of moisture on the system viscosity and dilution effects, and the literature reflects this with some reports of an increase in activation energy (E_A) with increasing a_w and others reporting a decrease in E_A with increasing a_w (*9*, *11*, *13*, *30*). In the presence of sufficient moisture, a solution is formed and degradation should therefore follow solution-phase pathways.

Since solution state vitamin C degradation would be expected to proceed at a faster rate than solid phase degradation, it can often be assumed for most systems that appreciable decomposition will occur only in the liquid phase (*31*). Therefore, the changes in vitamin C stability may be attributed to the amount of moisture present and correspondingly the ratio of vitamin C in the solid phase to the amount in the solution phase. However, while weight gain attributable to moisture uptake generally increased at higher RHs and corresponded to vitamin C loss, at some high RH and temperature storage conditions weight loss was observed toward the end of the storage time period. It has been reported that advanced degradation of ascorbate can result in release of volatile compounds. Shephard et al. (*29*) observed evolution of 1 mol of carbon dioxide per mol of ascorbic acid after storage at 60 °C and 5% v/w water for 42 days. The weight loss observed for both sodium ascorbate and ascorbic acid samples stored at 40 °C and high RH conditions could be due to advanced degradation resulting in loss of CO_2 thus affecting calculations of dissolved vitamin C content based on weight gain attributable to moisture uptake.

Degradation Kinetics. Vitamin C degradation has been reported to follow zero-order (*10*), first-order (*9*, *13*, *14*), and second-order kinetics (*15*). However, kinetics are commonly reported for high a_w foods or ascorbic acid solutions. Therefore, these kinetic models may not be applicable to a powder system where moisture uptake changes over time. In this study, vitamin C degradation was best described as a first-order reaction for both ingredient forms when total vitamin loss at the end of storage was at least 50%, based on the goodness of fit of a plot of $\ln(A/A_0)$ versus time (*32*). While others have reported zero-order

degradation of solid state vitamin C in the presence of small amounts of moisture (*2*), when less than 50% of the starting amount has degraded it is difficult to determine reaction order (*6*, *33*). Difficulty in distinguishing between reaction orders during solid state degradation of ascorbic acid has been reported by others (*34*, *35*). Generally vitamin C loss has been shown to follow first-order kinetics (*13*, *14*, *36*), and this holds true in this study at high RHs and where >50% degradation occurred. Additionally, at more extreme conditions (85% RH and 40 °C), a better fit to a first-order reaction was observed for ascorbic acid, even though degradation did not exceed ~14%. Obtaining good kinetic data for degradation in the solid state is difficult due to the complexity of solid state reactions compared to those occurring in solution (*25*). Especially for multiphase systems, such as occurs here, the heterogeneity and possibility for several competing reactions makes shelf life predictions difficult. Reaction rates and mechanisms can differ between dry powder systems, solutions, and concentrated solutions in contact with remaining solid, and the phase transformation induced by deliquescence and lack of steady state conditions as this process occurred likely contributed to the poor fit of kinetic models in this study. Extending the length of the study and collecting more data points for vitamin C stability could improve the fit of the kinetic models, especially for conditions in which loss is low.

At lower RH conditions where moisture uptake was minimal, visual observations indicated that sodium ascorbate had caked but appeared as a dry powder. Similarly, for ascorbic acid no liquid was present and appearance was described as dry crystals. Therefore, at these conditions it appears that both forms of vitamin C are in the solid state, and solid state degradation kinetics would be expected. Previously, a rate constant for solid state ascorbic acid degradation was determined (*25*). When 500 μL of water was added to the dry ascorbic acid, though, kinetic and thermodynamic parameters followed those expected for complete solution state reactions (*25*). This suggests that solid and solution state vitamin C shelf life could not be predicted by the same model. The amount of vitamin C in the solid and solution phases in this study is continually changing, and predicting storage life for dry powders undergoing moisture sorption and deliquescence is therefore difficult.

Shelf Life Models. Decreases in shelf life occurred for both vitamin C forms with increasing temperature (at constant RH) and a_w (at constant temperature), and Q_{10} and Q_A (*9*, *40*) values were consistent with stability observations. Generally ascorbic acid was more stable across RH (due to its reduced solubility and greater RH_0) than sodium ascorbate. Examination of the combined effect of temperature and a_w on vitamin C stability revealed a trend of further reduction in shelf life with increasing a_w as temperature was increased from 25 to 40 °C. Since solubility, and therefore RH_0, can be altered at higher temperatures, this trend is not unexpected (*39*).

Theoretically, each vitamin C form should exist as a saturated solution at its respective RH_0. As RH increased above the deliquescence RH, a more dilute solution should form, while below RH_0 the vitamin would be expected to remain in the solid state. Comparing rate constants and reaction orders for degradation of solid state, solution state, and saturated solutions of each vitamin C form highlights differences between solid and solution state degradation. Vitamin C degradation involves a complex series of reactions including an oxidation step followed by a slower hydrolysis reaction. The decomposition rate for the initial phase is dependent on the concentration of oxygen in the system (*37*). The presence of moisture would increase both the amount of dissolved oxygen available to participate in the oxidation step and water for the hydrolysis reaction, thus it is unsurprising that reaction rates increased as greater moisture was present in the system.

Perhaps a better explanation for the observed differences in vitamin degradation is a model proposed by Leeson and Mattocks (*38*). In this model, water can be assumed to be in the bulk phase when the number of moles of water in the system exceeds the number of moles of solid and degradation is mainly accounted for by decomposition in this phase (*38*). The amount of vitamin dissolved in these systems would determine total decomposition by solution kinetics (*31*). In other words, the reaction rate constants and stability predictions would be proportional to the amount of moisture present in the system. Carstensen and Pothisir (*34*) applied this concept in developing a model relating the overall decomposition rate of solid state *p*-aminosalicylic acid in the presence of moisture to the sum of the solid and solution state rate constants times the respective amounts of vitamin present in each phase. Recently, Guerrieri and Taylor (submitted) proposed a related model, whereby solid state reaction rate constants could be calculated from reported solution phase reaction rates and the weight percent of water present, with good agreement between predicted and observed results. While these models would be useful for studying reaction kinetics, additional data would be necessary to determine observed rate constants for both the solid and solution states in order to apply this model to vitamin C.

Generally, a strong relationship between moisture content and vitamin stability was observed. In this study, conditions where excess moisture was present resulted in appreciable vitamin C loss. Moles of water exceeded moles of sodium ascorbate after 8 weeks of storage at 75% RH and 35 and 40 °C, 85% RH at 25 and 40 °C, and 98% RH at 25 and 40 °C. Excess moles of water were present after 8 weeks of storage for ascorbic acid stored at 98% RH and 25 and 40 °C. Stability was enhanced for vitamin C samples where more moles of solid were present than moles of water. A better method for predicting vitamin C shelf life could be determination of amount dissolved versus solid remaining. Based on the limited losses of vitamin C during storage at RHs where drier conditions were maintained, it could be assumed that solid state degradation of vitamin C is of minor importance compared to the extensive losses observed under conditions of rapid moisture uptake. Previously, solid state and solution state ascorbic acid degradation rate constants were reported as 4.1×10^{-6} s^{-1} and 1.8×10^{-4} s^{-1}, respectively (*25*, *28*). The presence of moisture to aid in the hydrolysis step of vitamin C degradation is necessary for rapid deterioration, while oxidation of vitamin C under low moisture conditions does not appear to result in appreciable vitamin losses. However, the occurrence of some degradation while still in solid form cannot be discounted, and may contribute to the poor fit of the observed degradation to simple kinetic models.

These results highlight the importance of controlling storage conditions to ensure that moisture uptake resulting in an excess of water does not occur as a critical prevention method for maintaining vitamin C stability. Deliquescence resulted in significant moisture sorption by the vitamin samples and subsequent rapid deterioration. Additionally, the importance of improving understanding of combined temperature and RH effects on vitamin phase transformations and ultimate shelf life, as these two environmental factors can act synergistically, is emphasized. Furthermore, formulation would influence the impact of RH on reaction rates providing justification for extending understanding obtained from this simplistic model to more complicated powder systems. Improvements to vitamin C shelf life models could be made by better understanding degradation rates related to the amount of vitamin in solution. Plotting vitamin C dissolved

versus vitamin C remaining with incremental increases in the amount of vitamin in the solution phase would elucidate the relationship between moisture uptake and solution degradation kinetics, as solid state vitamin C was generally stable as long as moisture uptake was limited.

In summary, both RH and temperature impacted vitamin C degradation, with RH having a more profound effect. Additionally, the two storage conditions appeared to act synergistically, with higher temperatures resulting in decreased vitamin C stability when stored above a certain RH. Generally, vitamin C stability decreased dramatically near or above RH_0 for both vitamin forms. At higher temperatures, enhanced vitamin C solubility resulted in a reduction in RH_0 and promoted vitamin dissolution and subsequent degradation. Shelf life plots, Q_{10}, and Q_A values demonstrated that both vitamin C forms were sensitive to increases in a_w, and a_w had a greater influence on stability of sodium ascorbate and ascorbic acid at higher temperatures. It also appears that an excess of water in the vitamin C system induces deterioration and increases in loss kinetics. Results of this study also demonstrate that solid state and solution state degradation pathways and kinetics may differ for vitamin C, complicating modeling of this reaction. More studies on solid and solution state degradation would be needed to determine the solution phase rate constants and the occurrence of variable degradation pathways. Separate parameters may need to be determined for RH ranges where solid state degradation predominates, and those where most of the solid is dissolved. Degradation of vitamin C that is then in both the solid state and solution phase can be compared to these processes. The importance of the presence of moisture and dissolution of vitamin C to overall stability is evident.

ABBREVIATIONS USED

RH, relative humidity; RH_0, deliquescence point of an individual crystalline ingredient.

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