Ultraviolet-Induced Oxidation of Ascorbic Acid in a Model Juice System: Identification of Degradation Products

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JUNE 2011

Impact Factor: 2.91 · DOI: 10.1021/jf201000x · Source: PubMed

4 AUTHORS, INCLUDING:

Rohan Tikekar
University of Maryland, College Park
33 PUBLICATIONS **222** CITATIONS

SEE PROFILE

Luke Frederick Laborde
Pennsylvania State University
34 PUBLICATIONS **325** CITATIONS

SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Ultraviolet-Induced Oxidation of Ascorbic Acid in a Model Juice System: Identification of Degradation Products

Rohan V. Tikekar, Ramaswamy C. Anantheswaran, Ryan J. Elias, and Luke F. LaBorde*

Department of Food Science, Pennsylvania State University, University Park, Pennsylvania 16802, United States

ABSTRACT: Degradation products of ultraviolet (UV-C, 254 nm) treated ascorbic acid (AA) are reported. Analysis by high-performance liquid chromatography−mass spectroscopy (HPLC-MS) conducted in a 0.5% malic acid model juice system (pH 3.3) demonstrated increased degradation of AA above untreated controls with concomitant increases in dehydroascorbic acid (DHA) and 2,3-diketogulonic acid (DKGA) levels. Electron spin resonance (ESR) spectroscopy studies, conducted in phosphate buffer (pH 7.0) to increase detection sensitivity, demonstrated that ascorbyl radical ($AA^{\bullet}$) formation occurs simultaneously with AA degradation. Consistent with a previous study in which UV treatments were shown to accelerate dark storage degradation, $AA^{\bullet}$ radicals continued to form for up to 200 min after an initial UV treatment. Results from this study suggest that the mechanism for UV-induced degradation is the same as the general mechanism for metal-catalyzed oxidation of AA in juice.

KEYWORDS: ultraviolet radiation processing, ultraviolet light processing, ascorbic acid, apple juice, nonthermal food processing, electron paramagnetic resonance spectroscopy

INTRODUCTION

Ascorbic acid (AA) is an endogenous component of many plant-based foods and is often added as a vitamin C supplement or to serve as an antioxidant to minimize losses in color, flavor, and nutrients during processing and storage.[1,2] AA degradation in foods primarily occurs through an initial one-electron transfer from ascorbyl anions to yield ascorbyl radicals. This reaction is followed by a subsequent one-electron transfer to yield dehydroascorbic acid (DHA).[2] Because both reactions are reversible, AA and DHA have vitamin C activity in the human body. DHA, however, is a relatively unstable compound and is rapidly and irreversibly hydrolyzed to 2,3-diketogulonic acid (DKGA), which has no vitamin C activity. AA degradation in foods is influenced by matrix pH, dissolved oxygen concentration, the presence of transition metal catalysts, and oxidative enzyme activity.[1]

AA degradation in foods is accelerated by exposure to visible light and polychromatic UV radiation, particularly when photosensitizing agents are present, including riboflavin,[3,4] food dyes,[5] methionine,[6] and some sweeteners.[7] Juice and juice model system studies have shown that exposure to disinfectant UV-C (254 nm) light also increases AA degradation.[8−10] We recently reported the effect of individual juice constituents on UV-C-induced AA degradation.[11] Degradation occurred more rapidly at higher UV dose levels, pH levels, and malic acid concentrations and more slowly at higher solution absorbance levels. Among the sugars tested, the addition of fructose at levels typically found in apple juice increased AA degradation, whereas glucose and sucrose did not. Following UV-C treatment, AA degradation continued for up to 30 h during dark storage and occurred more rapidly after a higher initial UV dose levels and at a higher storage temperature.

Because the ascorbyl radical is a terminal radical produced by the reaction of AA and reactive oxygen species (e.g., superoxide, perhydroxyl, hydroxyl radicals, singlet oxygen), it is a useful marker of oxidative stress in biological systems.[12] The relatively long half-life of ascorbyl radicals (ca. 50 s) makes it easily detectable by electron spin resonance (ESR) spectroscopy without the need for spin trapping techniques.[12] ESR has, therefore, been used extensively to study ascorbyl radical formation in biological systems[13−15] including animal[16] and plant tissues.[17] However, to our knowledge, there are no ESR studies showing the effect of UV-C radiation on ascorbyl radical formation in foods or food model systems.

Ultraviolet (UV-C) processing is an emerging alternative to heat pasteurization for reducing microbial levels in food products. It is particularly suited to juice processing because it is comparatively lower in cost, has minimal effects on product flavor, and is adaptable to continuous processing methods.[18−20] Significant reductions in spoilage[10,21,22] and human pathogenic microorganisms[22−24] have been demonstrated through the use of UV processing. The incentive for commercialization of UV juice technology increased when the U.S. Food and Drug Administration mandated a 5-log pathogen reduction requirement for all juice processors.[25] Many smaller scale operations that did not pasteurize before the regulation came into effect have since purchased UV treatment equipment, most notably the CiderSure UV system (FPE, Rochester NY), which has been proven effective in achieving the pathogen reduction standard.[23,26] Understanding the chemical reactions in foods subjected to UV-C processing is, therefore, important as this technology moves forward.

In earlier studies on the photodegradation of AA in solution, the wavelengths used were often combinations of visible light and UV radiation or otherwise not adequately defined. The objective of this study was to determine the effects of UV-C (254 nm) disinfectant radiation, currently used in commercially available juice treatment systems, on AA degradation and product formation using ESR and HPLC-MS detection.

Received: March 11, 2011
Revised: June 20, 2011
Accepted: June 24, 2011
Published: June 24, 2011

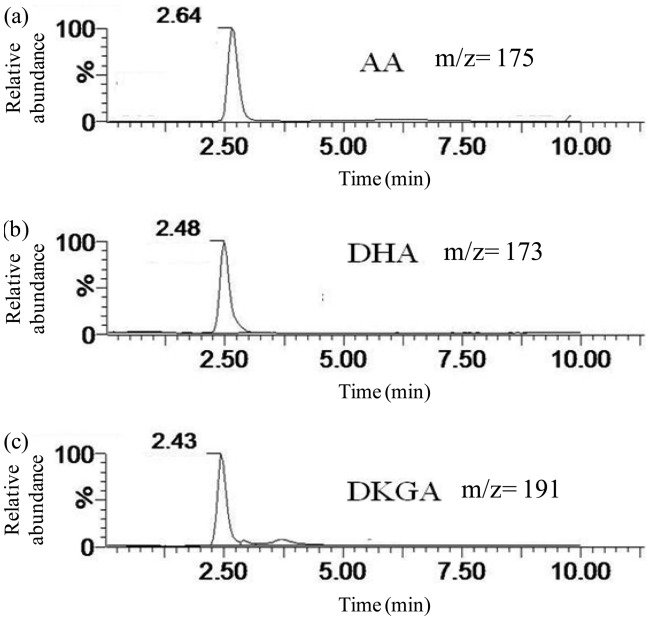

Figure 1. Representative HPLC-MS (negative single ion monitoring mode) chromatogram of products formed after UV exposure of AA in 0.5% malic solution (pH 3.3) for 3 h: (a) AA; (b) DHA; (c) DKGA. (AA_0 = 400 mg/L.)

MATERIALS AND METHODS

Reagents. DHA, ethylenediaminetetraacetic acid (EDTA), formic acid, malic acid, and mono- and dibasic sodium phosphate were purchased from Sigma-Aldrich (St. Louis, MO). AA and acetonitrile (HPLC grade) were purchased from Fisher Chemicals (Pittsburgh, PA). 2,2′-Azobis(2-methylpropanimidamide hydrochloride) (AAPH) was obtained from Wako Chemicals (Richmond, VA).

Sample Preparation and UV Treatment. AA was added to 37.3 mM (0.5%) malic acid (pH 3.3) or 10 mM phosphate buffer (pH 7.0). Treatments were carried out by adding AA solution (30.0 mL) to an uncovered plastic Petri dish (100 mm × 10 mm) and exposing it to UV radiation at appropriate time intervals. At each sampling point, 0.6 mL of treated solution and an equal volume of untreated solution that had been held in the dark for the same time interval were analyzed. Samples for HPLC-MS analysis were immediately frozen at −15 °C and analyzed the following day. Samples for ESR analysis were analyzed immediately.

All experiments were carried out using the benchtop batch collimated beam UV reactor described earlier by Tikekar and others.[11] The reactor consisted of three UV-C lamps (254 nm, 10 W, Atlantic Ultraviolet Inc., Hauppauge, NY) mounted within a shielded horizontal cylindrical holder fitted over a vertical tube (100 mm diameter × 100 mm length). Collimation was achieved by painting the inside surface of the vertical tube with UV-absorbing black paint. On the basis of the length of the tube, the calculated maximum incident angle was no greater than 20°. Incident intensity (1.4 mW/cm^2) was measured by placing a radiometer (model UVP-J225, UVP LLC, Upland, CA) at the bottom of the tube at a length equal to the distance between the light source and the surface of the sample. Variation of incident intensity over the entire sample surface area was <1%. This slight error was neglected because the sample was continuously stirred with a mechanical stir bar (300 rpm).

HPLC and MS Analysis. HPLC-MS was used for the simultaneous determination of AA, DHA, and DKGA concentrations in the malic acid reaction medium. Analyte separation was achieved using a reverse phase/cation exchange column (Primesep-D, 4.6 mm × 150 mm, particle size = 5 μm, SIELC Inc., Prospects Heights, IL). The HPLC system (Shimadzu Scientific Instruments, Columbia, MD) consisted of a controller (SCL-10A), a binary pump system (LC-10AD), a UV−visible detector (SPD-10A), and an autosampler (SIL-10AD). The mobile phase consisted of water/acetonitrile/formic acid (95:5:0.95; v/v/v) adjusted to pH 1.8 (solvent A) and acetonitrile (solvent B). A linear gradient mode was used to achieve the following mobile phase conditions: 100% A/0% B at 0 min to 80% A/20% B in 10 min. The flow rate was maintained at 1 mL/min. The MS system (Micromass Quattro Micro triple quadrupole, Waters Inc., Milford, MA) was run in negative single ion monitoring mode to identify and quantify DHA (m/z 173), AA (m/z 175), and DKGA (m/z 191). MS operating parameters were as follows: capillary voltage, 3.2 kV; cone voltage, 25 V; source temperature, 100 °C; desolvation temperature, 250 °C; gas flow, 500 L/h; cone gas flow, 50 L/h. The flow rate to the MS was split 1:4 to achieve a 250 μL/min flow. A representative HPLC-MS chromatogram is shown in Figure 1. Quantification of AA and DHA acid concentrations was based on external standard curves prepared from authentic standards. Because an authentic DKGA standard was not available, relative changes in DKGA concentration were plotted.

The HPLC method previously developed in our laboratory[11] was used to determine reductions in AA concentration in pH 7.0 phosphate buffer while simultaneous formation of ascorbyl radicals was followed using ESR. A Waters HPLC system consisting of a pump (600), an autosampler (71P), and a photodiode array (PDA) detector (2998) was used. Separation was achieved using the same Primesep-D column described above. The isocratic mobile phase consisted of a water/acetonitrile/formic acid (95%) mixture (95:5:0.095 v/v/v) adjusted to pH 1.80 with HCl.

ESR Spectroscopy. A Bruker-Biospin e-Scan R X-band ESR system (Bruker Biospin Inc., Billerica, MA) was used for ascorbyl radical measurements. Samples treated with UV in phosphate buffer (pH 7.0) were analyzed after UV treatment by loading (ca. 1 mL) into 19-bore quartz cells and placing them within the ESR cavity. Contrary to the use of pH 3.3 malic acid for the determination of AA, DHA, and DKGA, ESR experiments were performed in pH 7.0 phosphate buffer because the sensitivity of the ESR system was not sufficient to detect a signal response above noise in both untreated and UV-treated samples in malic acid. The settings for the detection of ascorbyl radicals were as follows: center field, 3488.225 G; sweep width, 20 G; static field, 3468.236 G; frequency, 9.77 GHz; attenuator, 2.0; power, 37.86 mW; modulator frequency, 86 kHz; modulation amplitude, 0.69 G; modulation phase, 1.08°; offset, 1%; time constant, 327.68 ms; conversion time, 20.48 ms; number of scans, 16. Because no significant line widening was observed (av = 3.51 ± 0.19 G), peak intensity values were determined from peak-to-peak heights determined according to the equation

$$\text{peak intensity} = (h_1 + h_2)/2 + |h_3| \quad (1)$$

where h_1 and h_2 are the positive spectral arbitrary unit (AU) values for the high-field peaks and h_3 is the absolute AU value for the low-field peak (Figure 2). Relative peak intensities, calculated to minimize signal response variation between runs, were calculated by dividing treatment peak intensities at each time interval by the average peak intensity for untreated controls.

The method described by Buettner and Jurkiewicz was used to generate a representative ESR ascorbyl radical spectrum.[12] Ascorbyl radicals were generated by mixing 250 mM AAPH in ultrapure water (Sigma-Aldrich) with an equal volume of 4 mM AA. The addition of a spin trap was not necessary due to the relatively long half-life of ascorbyl radicals.[12] Spontaneous formation of ascorbyl radicals by metal-catalyzed oxidation reactions was minimized by adding 0.5 mM EDTA to reaction solutions.[27] The lower limit of detection for peak heights was 3.0 × 10^5 AU.

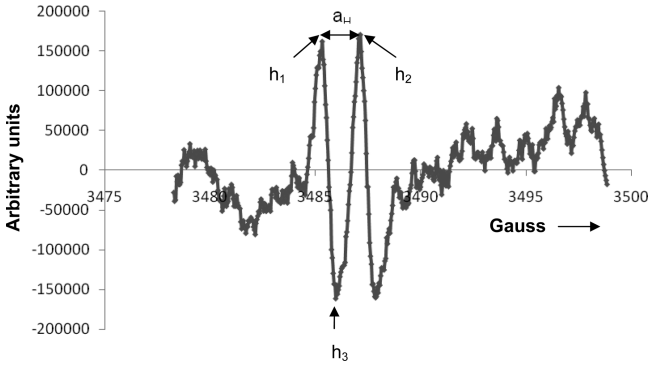

Figure 2. Representative ESR spectrum for ascorbyl radical ($AA^{\bullet}$) generated in pH 7.0 phosphate buffer. a_H = hyperfine coupling constant. h_1, h_2, and h_3 = AU values for peaks as shown.

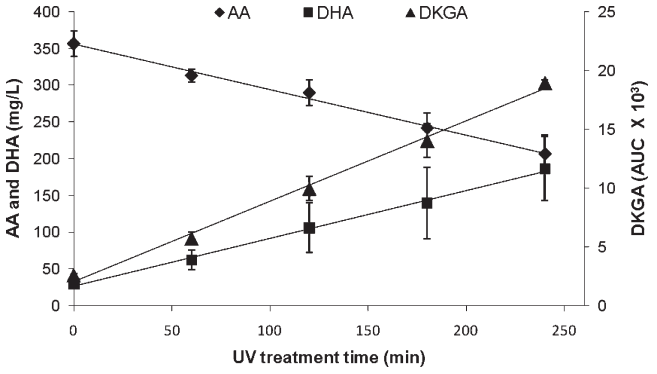

Figure 3. Degradation of AA (C_0 = 356 ± 18 mg/L) and formation of DHA and DKGA in 0.5% malic acid (pH 3.3) after exposure to UV light. Each data point represents the average of three replicate treatments ± standard deviation.

RESULTS AND DISCUSSION

AA Degradation and Products Formation in Malic Acid Buffer. We hypothesized that, similar to the known oxidative degradation pathway of AA,[1] UV degradation will yield DHA and DKGA. Therefore, a HPLC-MS experiment was conducted to identify these degradation products. UV-induced AA degradation and simultaneous formation of DHA and DKGA were studied in a 0.5% malic acid (pH 3.3) reaction medium. This medium was chosen because it was identical to that used in our earlier apple juice model system study in which the effects of individual juice chemical constituents on UV-induced AA degradation were determined.[11] AA concentrations in UV-treated samples decreased significantly ($p \leq 0.05$) from 356 ± 18 to 206 ± 26 mg/L after 4 h. At the same time, DHA concentration significantly increased from 30 ± 4 to 186 ± 44 mg/L as DKGA levels, measured by peak area, increased 5.5-fold (Figure 3). AA levels in untreated samples held in the dark decreased only slightly (<5%) over the course of the experiment, which is in agreement with our earlier study.[11] Although the absence of a DKGA standard prevents comparisons of actual concentrations for all compounds, the data strongly suggest that the degradation mechanism for UV-induced AA degradation is the same as that for the general oxidative degradation mechanism for AA.[1]

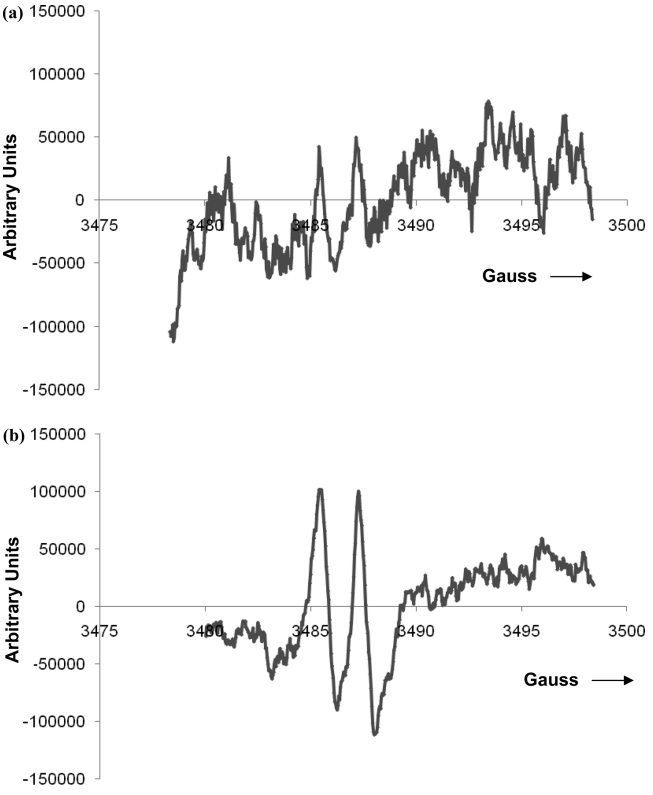

Figure 4. ESR spectra for ascorbyl radical ($AA^{\bullet}$) generated in pH 7.0 phosphate buffer before (a) and after (b) UV treatment for 60 min.

Because the general mechanism for DHA formation includes a one-electron transfer from the ascorbyl anion to form an ascorbyl radical, we attempted to confirm ascorbyl radical formation during UV treatments using ESR spectroscopy. However, the sensitivity of the ESR system was not sufficient to detect a signal response above noise in both untreated and UV-treated samples prepared in 0.5% malic acid buffer (pH 3.3). Therefore, the neutral pH phosphate buffer system of Buettner and Jurkiewicz was used to follow radical formation during UV treatments and post-UV dark storage.[12]

UV-Induced Ascorbyl Radical Formation in Phosphate Buffer. The reference ESR spectrum of AA after the addition of AAPH, a known ascorbyl radical ($AA^{\bullet}$) producer shown in Figure 1, shows a characteristic doublet between 3484 and 3487 Ga with a hyperfine coupling constant (a_H) of 1.8. This value is in agreement with that (a_H = 1.88) reported by Pietri and others for the ascorbyl radical.[13] The ascorbyl radical ($AA^{\bullet}$) spectra generated before and after UV treatment of AA in phosphate buffer for 60 min is shown in panels a and b, respectively, of Figure 4. The coupling constant (a_H) in this system was 1.8, thus confirming that the observed doublet represents the signal for the ascorbyl radical. In contrast to the poor peak intensity response in pH 3.3 malic acid (data not shown), a strong signal for ascorbyl radicals was observed in pH 7.0 phosphate buffer. It is likely that higher detectable levels of ascorbyl radicals observed at pH 7.0, compared to pH 3.3, are due to the fact that the ascorbate oxidation occurs more rapidly at higher pH values.[1] Because an ascorbyl radical formation step necessarily precedes formation of DHA and DKGA,[1] it is likely that AA in pH 3.3 malic acid exposed to UV light also results in the formation of ascorbyl radicals, although at comparatively lower concentrations.

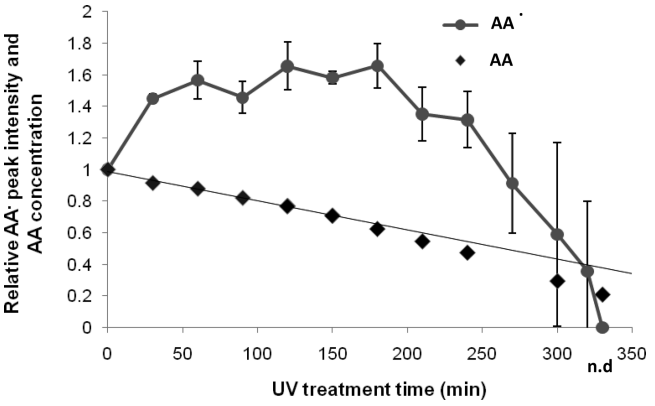

Figure 5. AA (C_0 = 450 mg/L) degradation and ascorbyl radical ($AA^{\bullet}$) formation in phosphate buffer following UV treatment. AA data points represent the average of two replicate treatments. $AA^{\bullet}$ data points represent the average of three replicate treatments ± standard deviation. n.d. = below detection limit.

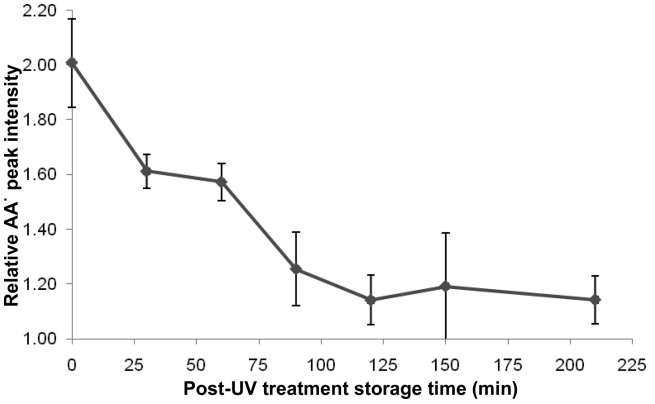

Figure 6. Relative ascorbyl radical ($AA^{\bullet}$) peak intensities after UV treatment of AA (C_0 = 600 mg/L) in pH 7.0 phosphate buffer for 60 min followed by dark storage for up to 200 min. Each data point represents an average of three replicate treatments ± standard deviation.

UV-Induced AA Degradation and Ascorbyl Radical Formation in Phosphate Buffer. AA degradation (C_0 = 450 mg/L) and concomitant ascorbyl radical ($AA^{\bullet}$) formation in pH 7.0 phosphate buffer during UV treatments for up to 330 min are shown in Figure 5. AA degradation followed zero-order kinetics for up to 150 min (r = 0.991), after which the reaction rate increased. This is in agreement with results from our earlier study,[11] in which the rate acceleration effect was attributed to greater penetration of UV radiation into the reaction solution as AA levels decreased and possibly generation of reactive side products. Relative peak intensities for ascorbyl radicals significantly ($p \leq 0.05$) increased between 0 and 60 min of UV exposure, after which no significant ($p > 0.05$) change occurred up to 180 min. At longer treatment times, AA levels became the limiting factor in radical formation as evidenced by relative peak intensities that significantly ($p \leq 0.05$) decreased for up to 330 min. Spectral intensities for samples held in the dark during the same time intervals did not change significantly ($p > 0.05$). Therefore, the data show that the amount of AA remaining in treated samples was sufficient to generate ascorbyl radicals at levels above untreated controls (relative peak intensity > 1.0) for up to 270 min. At longer times, AA levels decreased to an extent such that ascorbyl radical levels were below that of untreated controls (<1.0).

Post-UV Treatment Degradation of AA in Phosphate Buffer. Figure 6 shows relative peak intensities for ascorbyl radicals ($AA^{\bullet}$) for up to 210 min of storage following UV treatment of AA (AA_0 = 600 mg/L). As expected, radical peak intensities immediately after UV treatment (t = 0 min) were higher than untreated controls (>1.0). Levels then significantly ($p < 0.05$) decreased for up to 90 min, after which no further significant decreases occurred. Relative peak heights remained slightly above 1.0 during this period.

These results are consistent with our model system and juice studies[11] in which we reported that AA treated with UV light continued to degrade during dark storage. Ascorbyl radicals generated during UV treatment cannot explain this effect given their relatively short half-lives.[12] Spontaneous metal-catalyzed aerobic oxidation may partially explain AA decreases and persistent levels of ascorbyl radicals.[28] However, our earlier data that showed a significant positive relationship between initial UV treatment dose and dark storage degradation rate suggests that a more stable reactive compound was formed during UV exposure. Hydrogen peroxide, a byproduct of metal-catalyzed aerobic oxidation of AA,[1,28] is also a known photodegradation byproduct of AA[29] and has been shown to accelerate oxidation of AA in model systems[30] and in juice products.[31] It may therefore play an important role in the post UV treatment persistence of ascorbyl radicals observed in this study.

Conclusion. The results of this study provide insight into the effects of UV-C disinfectant radiation on AA loss and degradation product formation in juice systems. HPLC-MS and ESR data strongly suggest that UV-induced degradation of AA proceeds by a similar mechanism as that for the general metal-catalyzed oxidation pathway. Ascorbyl radicals generated upon exposure to UV light persist during dark storage for a period beyond that which would be expected given their known half-lives. Further studies are needed to determine if formation of stable intermediates, such as hydrogen peroxide, is enhanced during UV treatments and if this is related to continued AA degradation and ascorbyl radical formation during post-UV dark storage. In the general mechanism for aerobic oxidation of AA, DKGA is known to continue to degrade into other compounds including furfural and 2-furoic acid.[32] Although we observed that DKGA levels continued to increase within the UV treatment times studied, additional studies would be useful to determine if these and other end products are formed and if they have an effect on the quality of UV-processed juice products. Further research is also necessary to refine ESR techniques so that direct measurement of ascorbyl radicals under lower pH conditions in malic acid buffer or in juice systems can be determined.

AUTHOR INFORMATION

Corresponding Author

*Postal address: 442 Food Science Building, University Park, PA 16802. E-mail: lfl5@psu.edu. Phone: (814) 863-2298.

ACKNOWLEDGMENT

We thank Smaro Kokkinidou for her assistance in conducting HPLC-MS experiments.

REFERENCES

(1) Gregory, J. Vitamins. In *Food Chemistry*, 4th ed.; Damodaran, S., Parkin, K., Fennema, O., Eds.; CRC Press: Boca Raton, FL, 2008; pp 468−469.

(2) Liao, M. L.; Seib, P. A. Chemistry of L-ascorbic acid related to foods. *Food Chem.* **1988**, *30* (4), 289–312.

(3) Jung, M. Y.; Kim, S. K.; Kim, S. Y. Riboflavin sensitized photooxidation of ascorbic acid kinetics and amino acid effects. *Food Chem.* **1995**, *53* (4), 397–403.

(4) Vaid, F. H. M.; Shaikh, R. H.; Ansari, I. A.; Ahmad, I. Spectral study of the photolysis of aqueous thiamine hydrochloride and ascorbic acid solutions in the presence and absence of riboflavin. *J. Chem. Soc. Pakistan* **2005**, *27* (3), 227–232.

(5) Sidhu, D. S.; Sugden, J. K. Effect of food dyes on the photostability of aqueous solutions of L-ascorbic acid. *Int. J. Pharm.* **1992**, *83* (1−3), 263–266.

(6) Asker, A. F.; Canady, D.; Cobb, C. Influence of DL-methionine on the photostability of ascorbic acid solutions. *Drug Dev. Ind. Pharm.* **1985**, *11* (12), 2109–2125.

(7) Ho, A. H. L.; Puri, A.; Sugden, J. K. Effect of sweetening agents on the light-stability of aqueous solutions of L-ascorbic acid. *Int. J. Pharm.* **1994**, *107* (3), 199–203.

(8) Adzahan, N. Effects of ultraviolet treatment on water soluble vitamin retention in aqueous model solutions and apple juice. Ph. D. dissertation, Cornell University, Ithaca, NY, 2006.

(9) Koutchma, T.; Forney, L. J.; Moraru, C. I. UV processing effects on quality of foods. In *Ultraviolet Light in Food Technology − Principles and Applications*, 1st ed.; Koutchma, T., Forney, L. J., Moraru, C. I., Eds.; CRC Press: Boca Raton, FL, 2009; pp 103−123.

(10) Tran, M. T. T.; Farid, M. Ultraviolet treatment of orange juice. *Innovative Food Sci. Emerging Technol.* **2004**, No. 5, 495–502.

(11) Tikekar, R.; Anantheswaran, R.; LaBorde, L. Ascorbic acid degradation in a model apple juice system and in apple juice during ultraviolet light processing and storage. *J. Food Sci.* **2011**, *76* (2), H62–H71.

(12) Buettner, G. R.; Jurkiewicz, B. A. Ascorbate free-radical as a marker of oxidative stress − an EPR study. *Free Radical Biol. Med.* **1993**, *14* (1), 49–55.

(13) Pietri, S.; Seguin, J. R.; Darbigny, P.; Culcasi, M. Ascorbyl free radical − a noninvasive marker of oxidative stress in human open-heart surgery. *Free Radical Biol. Med.* **1994**, *16* (4), 523–528.

(14) Sato, H.; Tani, A.; Ikeya, M. Electron spin resonance of ascorbyl (vitamin C) radicals in synthetic $CaCO_3$ by UV irradiation. *Jpn. J. Appl. Phys., Part 1* **2003**, *42* (2A), 428–433.

(15) Skotland, T.; Ljones, T. Direct spectrophotometric detection of ascorbate free radical formed by dopamine β-monooxygenase and by ascorbate oxidase. *Biochim. Biophys. Acta* **1980**, *630* (1), 30–35.

(16) Jurkiewicz, B. A.; Buettner, G. R. Ultraviolet light-induced free radical formation in skin − an electron paramagnetic resonance study. *Photochem. Photobiol.* **1994**, *59* (1), 1–4.

(17) Hideg, E.; Mano, J.; Ohno, C.; Asada, K. Increased levels of monodehydroascorbate radical in UV-B-irradiated broad bean leaves. *Plant Cell Physiol.* **1997**, *38* (6), 684–690.

(18) Guerrero-Beltran, J. A.; Barbosa-Canovas, G. V. Review: Advantages and limitations on processing foods by UV light. *Food Sci. Technol. Int.* **2004**, *10* (3), 137–147.

(19) Koutchma, T. UV light for processing foods. *Ozone-Sci. Eng.* **2008**, *30* (1), 93–98.

(20) Tikekar, R.; LaBorde, L.; Anantheswaran, R. Fruit juices: ultraviolet light processing. In *Encyclopedia of Food, Agricultural and Biological Engineering*, 2nd ed.; Heldman, D., Ed.; Taylor and Francis: New York, 2010; Vol. 1:1, pp 675−680.

(21) Keyser, M.; Muller, I. A.; Cilliers, F. P.; Nel, W.; Gouws, P. A. Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Sci. Emerging Technol.* **2008**, *9* (3), 348–354.

(22) Murakami, E. G.; Jackson, L.; Madsen, K.; Schickedanz, B. Factors affecting the ultraviolet inactivation of *Escherichia coli* K12 in apple juice and a model system. *J. Food Process. Eng.* **2006**, *29* (1), 53–71.

(23) Donahue, D. W.; Canitez, N.; Bushway, A. A. UV inactivation of *E. coli* O157:H7 in apple cider: quality, sensory and shelf-life analysis. *J. Food Process. Preserv.* **2004**, *28* (5), 368–387.

(24) Koutchma, T.; Keller, S.; Chirtel, S.; Parisi, B. Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. *Innovative Food Sci. Emerging Technol.* **2004**, *5*, 179–189.

(25) U.S. Food and Drug Administration. Hazard analysis and critical control point (HAACP); procedures for the safe and sanitary processing and importing of juice; final rule. *Fed. Regist.* **2001**, *66*, 6137–6202.

(26) Quintero-Ramos, A.; Churey, J. J.; Hartman, P.; Barnard, J.; Worobo, R. W. Modeling of *Escherichia coli* inactivation by UV irradiation at different pH values in apple cider. *J. Food Prot.* **2004**, *67* (6), 1153–1156.

(27) Miller, D. M.; Buettner, G. R.; Aust, S. D. Transition metals as catalysts of autoxidation reactions. *Free Radical Biol. Med.* **1990**, *8* (1), 95–108.

(28) Khan, M. M. T.; Martell, A. E. Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. I. cupric and ferric ion catalyzed oxidation. *J. Am. Chem. Soc.* **1967**, *89* (16), 4176–4185.

(29) Lavoie, J. C.; Chessex, P.; Rouleau, T.; Migneault, D.; Comte, B. Light-induced byproducts of vitamin C in multivitamin solutions. *Clin Chem.* **2004**, *50* (1), 135–140.

(30) Deutsch, J. C. Ascorbic acid oxidation by hydrogen peroxide. *Anal. Biochem.* **1998**, *255* (1), 1–7.

(31) Ozkan, M.; Kirca, A.; Cemeroglu, B. Effects of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices. *Food Chem.* **2004**, *88* (4), 591–597.

(32) Yuan, J.-P.; Chen, F. Degradation of ascorbic acid in aqueous solution. *J. Agric. Food Chem.* **1998**, *46*, 5078–5082.