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Furan Formation in Sugar Solution and Apple Cider upon Ultraviolet Treatment

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Furan is a possible human carcinogen induced by thermal processing of food. While ultraviolet C (UVC) is used to decontaminate apple cider and to sterilize sugar solutions, it is unknown whether UVC induces furan formation in cider or solutions of its major components. This study was conducted to investigate the possible formation of furan by UVC in apple cider and in solutions of common constituents of apple cider. Our results showed that UVC treatment induced furan formation in apple cider, and the major source of furan was apparently fructose. UVC treatment (at incident doses up to 9 J/cm^2) of fructose solutions produced a higher amount of furan, while very low concentrations of furan were induced by UVC in glucose or sucrose solutions, and virtually no furan was induced by UVC from solutions of ascorbic acid or malic acid. When an isotope (d_4-furan) of furan was treated with UVC, d_4-furan was destroyed rapidly even at low doses in fructose solution, suggesting that the accumulation of furan is the balance between destruction and formation. The UV sensitivity of *E. coli* K12 (a surrogate of *E. coli* O157:H7) in two sources of apple cider was also determined. At UVC doses that could inactivate 5-log of *E. coli*, very low concentrations (<1 ppb) of furan were induced. Our results suggest that UVC could induce furan formation, but when used for the purpose of juice pasteurization, little furan was induced in apple cider.

KEYWORDS: apple cider; carbohydrates; fructose; furan; ultraviolet; pasteurization; UVC

INTRODUCTION

The presence of furan in processed foods is a concern because furan is listed as "reasonably anticipated to be human carcinogen" in the Department of Health and Human Services Report on Carcinogens (*1*) and is considered "possibly carcinogenic to humans" by the International Agency for Research on Cancer (*2*). In a recent survey, the U.S. Food and Drug Administration (FDA) found that furan is present in many thermally processed foods purchased from supermarkets, with furan levels of ~100 ppb in some of the foods (*3*). Apple juice as a baby food contained furan levels ranging from 2.5 to 8.4 ppb (*3*). Furan is formed from carbohydrates, ascorbic acid, fatty acids, and a mixture of all three upon heating (*4, 5*). Our recent studies suggested that ionizing radiation, a nonthermal processing technology, induced furan formation in fruit juices and their constituents (*5, 6*). The amounts of furan formed as a result of ionizing irradiation were generally in the low ppb range. Furan is formed from ascorbic acid and carbohydrates by ionizing irradiation. Both U.S. FDA and European Food Safety Authority are seeking data on furan (*7, 8*).

Apple cider has been implicated in several *E. coli* O157:H7 outbreaks since 1980 (*9*). In response to the outbreaks, the FDA (*10*) initiated regulations that required juice manufacturers to achieve a minimum 5-log reduction of the most resistant pathogen. UVC processing is a nonthermal disinfection technology that has been demonstrated to inactivate pathogens in apple cider (*11–15*). FDA amended the food additive regulations to allow the use of UVC treatment to reduce human pathogens in juice (*16*). There are a number of commercial UVC systems available for processing apple cider (*17, 18*). UVC has been successfully applied to increase the shelf life of apple cider without affecting quality (*19, 20*). In addition, UVC is also used to disinfect drinking water and soft drinks, deactivate dormant spores in sugar syrups, and sterilize sugar solutions (*21, 22*).

Even though UVC is currently used by some small apple cider processors to achieve a 5-log reduction of pathogens that is required by the FDA, it is unknown whether UVC treatment induces furan in apple cider. The objectives of this study were to investigate whether UVC induced furan in apple cider and solutions of its components and to determine furan levels in apple cider exposed to UVC at doses that would inactivate *E. coli* by 5-log.

MATERIALS AND METHODS

Chemicals and Materials. Furan (99%), d_4-furan (99%), D-fructose, D-glucose, DL-malic acid, and L-ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO). Commercial apple cider was obtained from an apple cider producer (Zeigler Beverage Co., Lansdale, PA).

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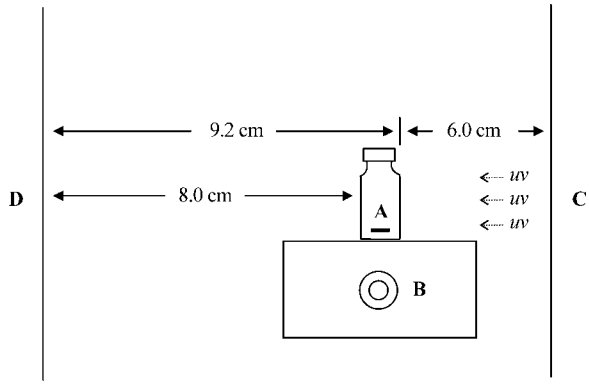

Figure 1. Diagram of UV treatment system used in the present study. The system consists of a quartz cuvette (A) with a magnetic stir bar, a stir plate (B), a UV source (C), and stainless steel wall (D).

Apples (cv. Gala) used to prepare fresh apple cider were purchased from a local supermarket. The ciders were either used in 3 days or stored at −20 °C for later use.

UVC Treatment. UVC was generated from eight model FG15T8 15 W germicidal fluorescent lamps (Buylighting.com, Burnsville, MN) mounted into a box (Ultra-Violet Prod. Inc., San Gabriel, CA). The low-pressure mercury-vapor lamps emit about 86% of their irradiation at 254 nm. The UVC box was placed into a biohood at ambient temperature (23 °C), and a Thermix Stirrer (model 120mR) stir plate (Fisher Scientific, Nepean, Ontario, Canada) was placed next to the UVC box (**Figure 1**). Each sample (1.5 mL) was placed into a 3 mL quartz cuvette (Spectrocell, Oreland, PA) containing a mini magnetic stir bar, and the cuvette was sealed using a septum and cap. The cuvettes had 1 cm light path with two polished sides that were UV-transparent. All samples were cooled to 5 °C on ice before being exposed to UVC unless otherwise stated. During UV treatment, quartz cuvettes containing the samples were set straight up on the stir plate on a marked line perpendicular to the UVC box. The samples were stirred at a speed setting of 2 during UV treatment. The quartz cuvettes allowed about 90% transmission of UVC. The measurement of UVC (at 254 nm wavelength) intensity at the same distance as the cuvettes were made using a UVX-25 radiometer (UVP Inc., Upland, CA) calibrated by the Optical Technology Division of the National Institute of Standards and Technology. The UV incident dose was calculated with the following equation: UV dose (mJ/cm^2) = irradiance (mW/cm^2) × exposure time (s) × transmittance factor. The transmittance factor is 0.9; i.e. about 90% of UVC was transmissible through the quartz cuvettes. Different doses of UVC were achieved by varying the exposure times.

Temperature of Apple Cider during UVC Treatment and Cooling. Temperature of apple cider was measured using a thermocouple inserted into the apple cider (1.5 mL, 0.5 cm above the stir bar) in the cuvette through the septum. The thermocouple (T cable type) was connected to an Acorn CL3512A thermometer (Omega Engineering Inc. Stamford, CT). The cuvette was cooled to ∼5 °C followed by exposure to UV for 20 min. The cider was stirred during UV treatment. Afterward, the cuvette was placed into ice–water as was done for all samples.

Formation of Furan from Apple Cider. Two sources of fresh apple cider were used in the study: a commercial unpasteurized cider from a local processor and a fresh cider made from "Gala" apples using a Champion MAR-48C juicer (Plastaket Manufacturing Co., Lodi, CA). Soluble solids content (Brix) and pH were 11.8 and 3.56, respectively, for the cider prepared in the laboratory and 11.1 and 3.47, respectively, for the commercial cider. The freshly prepared cider and commercial apple cider (1.5 mL), without further clarification, were then placed into the quartz cuvettes and cooled to 5 °C before UVC exposure for 1, 2, 4, 6, 8, and 10 min at irradiance of ∼10 mW/cm^2 at ambient temperature (23 °C). After the samples were cooled, d_4-furan as an internal standard was added to the cuvettes to a final concentration of 10 ppb. Furan was then analyzed and quantified.

Formation of Furan from Juice Solution of Constituents. The following solutions (5% each) were prepared in water and 0.25% malic acid: sucrose, fructose, glucose, and ascorbic acid. The pH values of the carbohydrate solutions were 5–6 and 2–3 in water and in malic acid, respectively, while pH of ascorbic acid in both water and 0.25% malic acid was 2.5. The samples (1.5 mL) were placed into the cuvettes and exposed to ∼9 J/cm^2 UVC at ambient temperature (23 °C). The dose response of furan formation in 5% fructose solution was also studied by exposing it to different doses of UVC. After treatment, the samples were spiked with the internal standard (d_4-furan) and analyzed for furan. Furan concentrations were calculated from standard curves that were established in corresponding solutions.

Destruction of d_4-Furan by UVC in Water, Sugar, and Ascorbic Acid Solutions and Juice. d_4-Furan solutions (100 ng/mL each) were prepared in deionized water or solutions of 5% fructose, sucrose, glucose, and ascorbic acid. Aliquots (1.5 mL) of the solutions were placed in the quartz cuvettes, and samples were cooled to 5 °C before UV-treated for ∼0.9 J/cm^2. Because d_4-furan was destroyed rapidly by UVC in 5% fructose, the dose response of d_4-furan in 5% fructose was also studied by exposing the samples to UVC treatment for various times.

Inactivation of *E. coli* K12 in Apple Cider. *Escherichia coli* K12 (ATCC 23716) was obtained from the American type Culture Collection (ATCC) (Manassas, VA). The bacterium was maintained on Tryptic Soy Agar (Remel, Lenexa, KS) at 4 °C. Prior to inoculation of product, the organism was cultured in Tryptic Soy Broth (Remel) with shaking at 37 °C for 16–18 h. Apple ciders either from the commercial source or processed freshly in our laboratory were inoculated from the stationary phase culture to give an approximately 6–7-log cfu/mL population. The inoculated apple cider (1.5 mL) was placed into 3 mL cuvettes and then exposed to ∼10 mW UVC for various times. After UVC treatment, appropriate dilutions of the product samples were made in Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria, CA). Duplicate samples (1 mL) were then pour plated with Tryptic Soy Agar (Remel) and the plates incubated at 37 °C for 24 h. Plates with 30–300 colonies were enumerated using a model 920 manual colony counter (Bantex, Burlingame, CA). The bacterial population was expressed as CFU/mL of apple cider. Data for each replicate were normalized against the control and plotted as the log reduction vs UVC dose.

Analysis of Furan and d_4-Furan. After UV treatment, all samples were spiked with d_4-furan. Furan and d_4-furan were analyzed as described earlier (*5*) with minor modification. Samples in the 3 mL cuvettes were incubated at 35 °C water bath for 25 min on a Corning heat/stir plate (Supelco, Bellefonte, PA) before a solid phase microextraction (SPME) fiber (85 μm Carboxen-PDMS) was inserted into the headspace of a vial. After 20 min of extraction time, the SPME fiber was inserted into the GC injection port at 240 °C and held for 5 min to desorb volatile compounds. Volatile compounds were separated by a Hewlett-Packard 5890/5971 GC-MSD (Agilent Technologies, Palo Alto, CA) equipped with a 3.5 m × 0.32 mm i.d. GasPro capillary column connected to a 30 m × 0.32 mm i.d., 0.1 μm DB-5 column (J&W Scientific, Folsom, CA) using a Universal Press-Tight Connector (Restek Chromatography Products, Bellefonte, PA). The temperature program of the GC oven was set to 50 °C for 2 min, increased to 130 °C at 10 °C/min, then to 250 °C at 15 °C/min, and held for 2 min at the final temperature. Helium was the carrier gas at a flow rate of 39 cm/s. The transfer line was held at 250 °C during the entire run. Furan and d_4-furan were identified by comparison of spectra of the sample compounds with those of standards and by comparing retention times of sample compounds with those of the standards. The *m*/*z* 39 and 68 ions and the ratio of 39/68 were used for the confirmation of furan, and *m*/*z* 68 was used as the quantifier. The *m*/*z* 41 and 72 ions and the ratio of 41/72 were used for the confirmation of d_4-furan, and *m*/*z* 72 was used as the quantifier. Furan was quantified using standard curve established in the individual matrix (apple cider or other juice constituents).

UVC Absorption of Apple Juice, Sugar, Ascorbic Acid, and Malic Acid Solutions. The UV absorbance (190–360 nm) of apple ciders and solutions of the three sugars, ascorbic acid, and malic acid were measured using a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Apple ciders were filtered through 0.45 μm Millipore (Billerica, MA) HV filter and then diluted 10 times. The sugar and malic acid solutions were undiluted while ascorbic acid was diluted to 0.001% (10 ppm) before measurement.

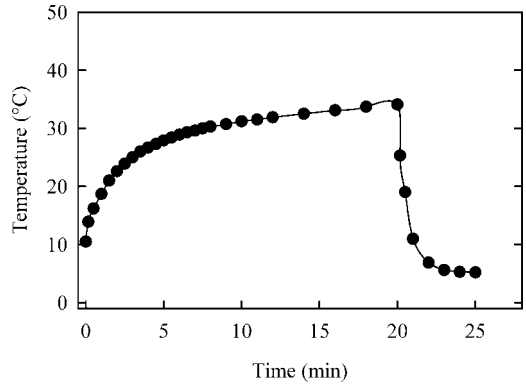

Figure 2. Changes in temperature of apple cider during UVC treatment and cooling.

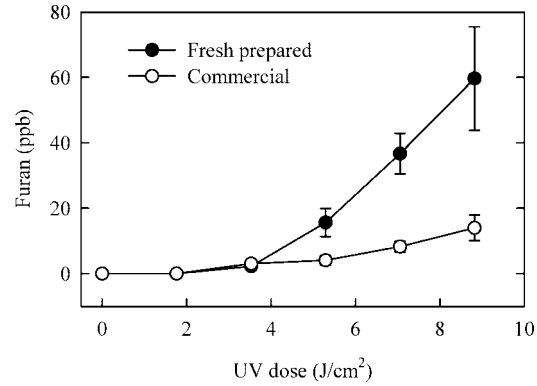

Figure 3. Formation of furan from freshly prepared and commercial apple ciders as a function of UVC dose. Vertical bars represent standard deviations ($n = 4$).

Statistical Analysis. The experimental design was a completely randomized design with at least four replicates. Data were subjected to statistical analysis using SAS Version 8 (SAS Institute, Cary, NC). Differences between treatments were analyzed by the least significant difference (LSD) test using the general linear model. In the figures, mean standard deviations are presented. When the difference between any two treatments is larger than the sum of standard deviations of the two treatments, it was always significant (LSD, $P < 0.05$). Only significant differences are discussed unless stated otherwise.

RESULTS AND DISCUSSION

UV Intensity. The UV intensity at the distance of the cuvette was measured first. After the UV lamps were turned on, UVC intensity did not approach the maximum immediately; i.e. the UVC intensity increased initially and then relatively stabilized after 2 min, reaching 9.5–10 mW/cm^2 (data not shown). However, there was no delay in reaching the maximum UVC intensity after the initial use if the UVC box was used again within 5 min. Therefore, the UVC box was used to treat samples after had been turned on for at least 2 min.

Temperature of Cider during UV Treatment and Cooling. The temperature of apple cider during UV treatment at the intensity of ~10 mW/cm^2 was measured. Before UVC treatment, juice temperature was about 5 °C (**Figure 2**). The temperature was about 10 °C once the sample was placed on stir plate. After the samples were exposed to UVC, juice temperature increased rapidly during the first 5 min. Then the increase slowed, reaching a temperature of 34 °C after 20 min. When the samples were cooled down in ice–water, juice temperature dropped to about 5 °C within 5 min.

Formation of Furan in Apple Cider. In the commercial fresh apple cider, UVC induced little furan at doses less than 3.5 J/cm^2 (**Figure 3**). Afterward, furan formation increased with UVC dose. At 8.8 J/cm^2, ~14 ppb furan was formed. Similarly, in freshly prepared apple cider, no furan was formed at the UVC dose of 1.8 J/cm^2. The furan formation, however, increased linearly ($R^2 = 0.99$) at a rate of 11 ppb per J/cm^2 UVC in the dose range of 3.5 and 8.8 J/cm^2, reaching about 60 ppb at 8.8 J/cm^2. The results showed that more furan was formed at higher doses (>3.5 J/cm^2) in the freshly prepared cider than in the commercial one.

Formation of Furan from Solutions of Sugars and Ascorbic Acid. Because we found UVC induced furan formation in apple cider, we then tried to determine the source(s) of furan. The major components of apple cider are sugars (fructose, sucrose, and glucose) followed by organic acids (mainly malic acid) (*23*). Apple cider contains a very low amount of ascorbic acid. UVC treatment of fructose solution produced a high amount of furan (**Figure 4**), and slightly higher furan was

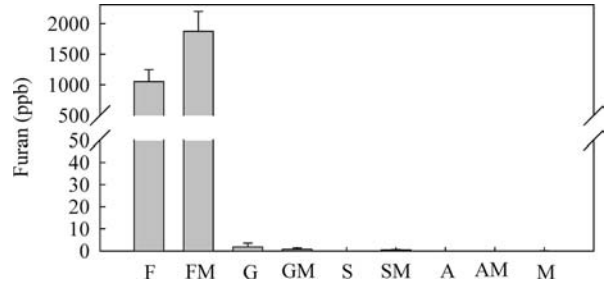

Figure 4. Formation of furan from sugars and ascorbic acid in aqueous and malic acid solutions. Solutions of 5% fructose (F), 5% fructose in 0.25% malic acid (FM), 5% glucose (G), 5% glucose in 0.25% malic acid (GM), 5% sucrose (S), 5% sucrose in 0.25% malic acid (SM), 5% ascorbic acid (A), 5% ascorbic acid in 0.25% malic acid (AM), and 0.25% malic acid (M) were exposed to ~9 J/cm^2 UVC at ambient temperature (~23 °C). Vertical bars represent standard deviations ($n = 4$).

produced from fructose solution prepared in 0.25% malic acid than that in water. Fructose in 0.25% malic acid had a lower pH than fructose solution in water, indicating the pH may have an effect on furan formation. Very low amounts of furan were produced from glucose or sucrose solutions prepared in either water or malic acid. Exposure of malic acid to UVC did not induce furan formation, and virtually no furan was formed from UVC treatment of ascorbic acid. Because the concentration (5%) of ascorbic acid was much higher than that commonly found in apple cider or any other foods, a low concentration (0.05%) of ascorbic was also UVC treated to study the concentration effect. UVC treatment of 0.05% ascorbic acid did not induce any furan formation either (data not shown).

Since furan was predominately formed from fructose, we then tested furan formation in fructose solution in responses to various doses. As dose increased, furan formation increased (**Figure 5**). Similarly to apple cider, there was an initial delay in the rapid formation of furan. Our earlier study (*5*) found that γ radiation induced furan formation in solutions of ascorbic acid, fructose, sucrose, and glucose. Heating also induced formation of furan from solutions of these compounds (*5*, *6*). The results in the present study suggest that furan was only formed in fructose solution, suggesting UVC has a different mechanism in inducing furan formation compared with γ irradiation and heating. In addition, pH had a dramatic effect on furan formation as a result of ionizing irradiation. For example, more than 5 times more furan was formed upon γ radiation in sugar solutions at low pH values (*2*, *3*) than at neutral pH values (5–6) (*5*). In the present study, the sugar solutions were prepared in water and 0.25% malic acid, with pH values of 5–6 and 2–3,

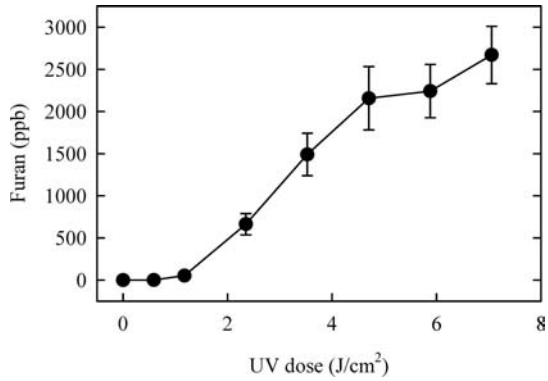

Figure 5. Formation of furan from 5% fructose solution as a function of UVC dose. Vertical bars represent standard deviations ($n = 4$).

respectively. However, the changes in pH had limited effect on UVC-induced furan formation in fructose solution, and no furan was formed upon UVC treatment in other solutions at either pH, once again suggesting a completely different mechanism of UVC-induced furan formation. Apple cider traditionally has a low pH (3–4) (*23*).

The simple sugars can be grouped into two types according to the ring structures: five-membered furanoses (furan like) (such as fructose) or six-membered pyranoses (such as glucose). The furanoses are less stable upon heating than pyranoses. For example, sucrose and glucose can be heated up to 100 °C, but fructose decomposes at a temperature as low as 60 °C (*24*). Our results showed that UVC treatment induced furan formation from fructose but not from glucose or sucrose, suggesting that fructose is more sensitive to UVC than the pyranoses. It will be interesting to study whether UVC treatment of furanoses other than fructose also induce furan formation.

Our results demonstrated that UVC treatment induced furan formation in fructose solution. Whether a significant amount of furan accumulates depends on radiation dose. If UVC is used to sterilize sugar solutions and syrup, accumulation of furan may occur. Further study is needed to determine the amount of furan (if any) that is formed after UVC sterilization of fructose solutions and syrups.

Reduction of d_4-Furan. During our development of furan analysis, we added d_4-furan as an internal standard prior to UVC treatment. We found that the d_4-furan was destroyed in fructose solution. Therefore, we analyzed the destruction of d_4-furan by UVC in different solutions and apple cider. There was little destruction of d_4-furan at the dose of ~0.9 J/cm^2 when d_4-furan in water, solutions of glucose, sucrose, ascorbic acid, or apple cider was UV-treated, but in fructose solutions, 88% of d_4-furan was destroyed (**Figure 6**). In water, less than 10% of d_4-furan was destroyed even at a dose of 9 J/cm^2. In fructose solution, all d_4-furan was destroyed at 9 J/cm^2. In a dose response study, we found that most d_4-furan was degraded even in the low dose (<0.1 J/cm^2) (**Figure 7**). It is possible that the degradation products of fructose may react with furan; the exact mechanism needs further study. Furan in aqueous solution is also sensitive to γ radiation (*5*). At low dose (1.0 kGy), all furan in water was destroyed by γ radiation in water. However, our results showed that UVC was not effective in reducing furan in water.

UVC Sensitivity of *E. coli*. Our results suggest that UVC treatment could induce furan formation. UVC is currently used by cider processors for inactivation of pathogens to meet the FDA requirement of at least 5-log reduction of the pertinent pathogen. It was unclear whether UVC treatment at a dose that achieves a 5-log reduction of the common pathogen (*E. coli* O157:H7) would induce furan formation in apple cider. To

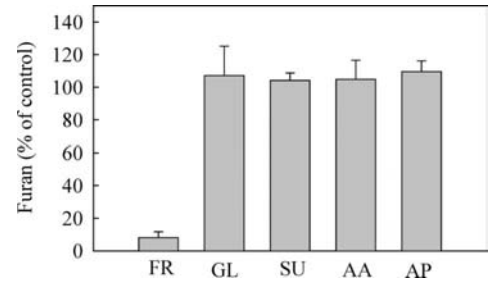

Figure 6. Reduction of d_4-furan in sugar and apple cider solution after exposure to 0.9 J/cm^2 UVC. d_4-Furan (100 ppb) in 5% solutions of fructose (FR), glucose (GL), sucrose (SU), and ascorbic acid (AA) as well as in freshly prepared apple cider (AP) was exposed to ~0.9 J/cm^2 UVC at ambient temperature (~23 °C). Vertical bars represent standard deviations ($n = 4$).

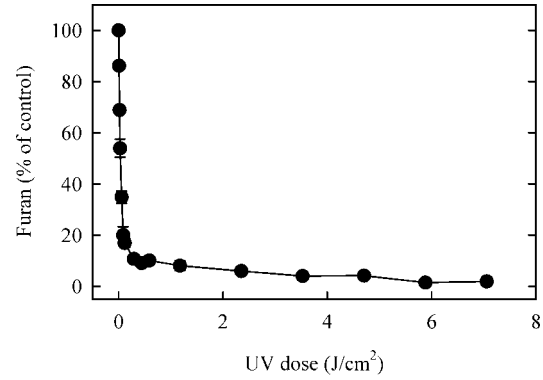

Figure 7. Reduction of d_4-furan in 5% fructose solution as a function of UVC doses. Vertical bars represent standard deviations ($n = 4$).

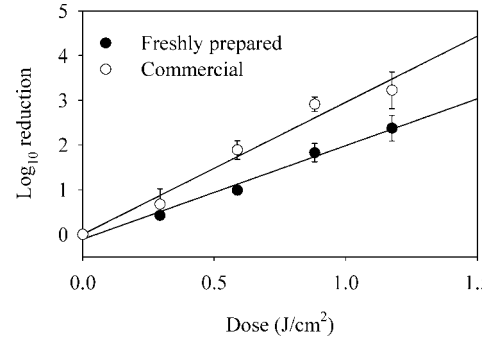

Figure 8. Relation between UVC dose and reduction of *E. coli* k12. The bacterium inoculated in either commercial apple cider or freshly prepared apple cider was exposed to various doses of UVC at ambient temperature. Vertical bars represent standard deviations ($n = 4$).

determine UVC sensitivity of *E. coli* under our conditions, we inoculated *E. coli* K12 (a surrogate of *E. coli* O157:H7) in two apple ciders and exposed them to different doses of UVC. The dose response of *E. coli* K12 to UVC appeared to be linear with R^2 of 0.97 and 0.98 in the commercial and fresh apple ciders, respectively (**Figure 8**). Our results suggest that *E. coli* in the freshly prepared apple cider was more resistant than that in the commercial cider. To achieve a 5-log reduction of *E. coli* in freshly prepared cider, a dose of 2.5 J/cm^2 is required, while in commercial cider 1.7 J /cm^2 was needed. At those doses, the furan levels would be 0.9 and 0.1 ppb in the freshly prepared and commercial ciders, respectively. In the FDA survey (*3*), furan levels in some thermally processed (canned) juices were in the range 2.5–8.4 ppb (*3*). Therefore, the amount of furan in UVC pasteurized apple ciders were much less than the thermally processed juices.

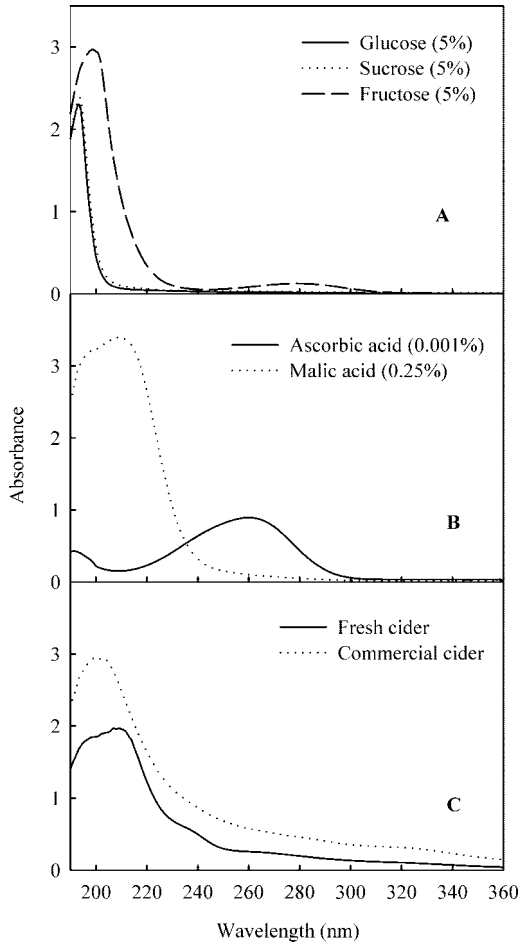

Figure 9. UV absorbance of 5% glucose, sucrose and fructose (A), 0.001% ascorbic acid and 0.25% malic acid (B), and diluted (10×) apple ciders (C).

One study (*12*) has shown that a dose of 61 mJ/cm^2 reduces *E. coli* O157:H7 by 5.4-log using a commercial UVC disinfection unit. Our study suggested that much higher UVC doses (1.6 and 2.5 J/cm^2) were required to achieve a 5-log reduction of the surrogate of *E. coli* O157:H7. In the present study, we used incident UV radiation to describe UV doses. It is not clear how much UV energy was actually absorbed by apple cider. Because of the long light path (1 cm) used in the present study, it is likely that most of UV energy was absorbed by the apple ciders. It may be useful to obtain the absorbed doses which require the prior determination of absorption coefficient of individual ciders. A commercial UVC disinfection unit utilizes a flow-through system that allows cold apple cider to be treated as a thin film. In the present study, we treated samples in cuvettes with stirring, and we used a lower UV intensity, which required much longer treatment time than commercial UVC processors to obtain the same log reduction of the pathogen. Even though we used a different UVC system, the study on inactivation of *E. coli* was conducted using the same setting as our furan formation study. Therefore, the amounts of furan induced by UVC at doses that achieved 5-log reductions could be estimated.

UV Absorption of Sugar, Ascorbic Acid and Malic Acid Solutions, and Apple Cider. The three sugars absorbed little UV in the range 240–360 nm although the fructose solution had higher UV absorbance at 260–280 nm than glucose and sucrose solutions. All three sugars had high absorbance around 200 nm (**Figure 9**A). Malic acid mainly absorbed UV at wavelengths less than 240 nm while ascorbic acid had a strong absorbance between 220 and 300 nm even at a very low concentration (0.001%) (**Figure 9**B). Apple ciders also had UVC absorbance at wavelengths below 240 nm (**Figure 9**C). Overall, the commercial apple cider had higher UVC absorbance than the freshly prepared cider. Analysis of the results revealed little correlation between UV adsorption and furan formation for the components in apple cider

The freshly prepared apple cider produced a higher amount of furan upon UVC treatment than the commercial cider. It is unclear what caused the difference in the amount of furan formation in response to UVC treatment. Composition of apple cider, turbidity, and clarity may play a role. Our results showed that furan is primarily formed from fructose. High amount of fructose in cider will increase furan formation due to UVC. Soluble solids content, which measures mostly sugar content, was higher (11.9 vs 11.1) in the freshly prepared cider than the commercial cider. The higher sugar content, presumably corresponding to higher fructose, may contribute to a higher amount of furan in the freshly prepared cider. The commercial processed apple cider may have more enzymatic browning occurring during processing compared to the cider prepared in the laboratory, resulting in a higher amount of pigments and absorbance in the UV range for the commercial apple cider. However, higher absorbance in the UV range is not necessarily an indicator of potential for furan formation. Apple cider with higher amounts of pigments (absorptivity) and suspended matter will reduce the penetration of UVC and may reduce the amount of UV absorbed by other components that produce furan. The apple cider prepared freshly in the laboratory had lower absorbance in the UVC range than the commercial cider (**Figure 9**C), which may permit the penetration of UVC treatment and increase furan formation.

Our results suggest that there was a delay in furan formation at low doses followed by a rapid linear increase in furan formation at high doses in both apple cider and fructose solution. It is unclear why little furan was detected after low dose UVC treatment. Perhaps temperature of solutions may play a role as temperature of samples was low initially and increased rapidly during the earlier period of UV treatment (**Figure 2**). We tested the furan formation in fructose solution with two different temperatures. Fructose solution at 23 °C formed slightly more furan upon UVC treatment than at 5 °C (data not shown). However, it could not totally account for the delay in furan formation. Initial sample temperature (5 °C vs 23 °C) had no significant effects on the UV-induced formation or destruction of furan in apple cider or fructose solution (data not shown). It appears that the accumulation of furan in apple cider is the result of the balance between furan formation and destruction. Our earlier study (*25*) showed that as γ radiation dose increased, the rate of furan reduction decreased while the rate of furan formation was maintained at a similar level, resulting in overall accumulation of furan. The rapid destruction by low-dose UVC may also contribute to the initial delay in the accumulation of furan occurring in apple cider and fructose solution.

All sugars and malic acid had low absorbance in the UV range (190–360 nm) although the absorbance of fructose was higher than other sugars (**Figure 9**A), which may contribute in part to the UVC-induced furan formation in fructose solution. On the other hand, ascorbic acid had very strong absorbance in the UVC range, but no furan was formed upon UVC treatment. Furan formation in the solutions did not always correlate with UVC absorbance.

The bactericidal effect of UVC is due to the destruction of nucleic acids, which absorb UVC at 250–260 nm (*26*). It

is unknown which part of the UV spectrum is most responsible for furan formation from fructose. More furan was formed from freshly prepared cider than from commercial cider while *E. coli* in the freshly prepared cider was more resistant to UVC than that in the commercial cider. Given the differences in furan formation and inactivation of *E. coli* between the two apple ciders, it is likely that UVC ranges other than 250–260 nm may be responsible for furan formation. Lower than 250 nm wavelength UVC will have higher energy and may induce more furan formation. On the other hand, the UVC lamps used in the present study emitted UVC mostly in the 250–260 nm range. Furthermore, turbidity, the types of apples used for the processing of ciders, and the different processing and handling conditions may also contribute to the differences between the two ciders in *E. coli* inactivation and furan formation.

In summary, our results showed, for the first time, that UVC could induce furan formation in apple cider, and fructose solution. However little furan was formed from solutions of sucrose, glucose, ascorbic acid, or malic acid, suggesting that fructose was likely responsible for the accumulation of furan in apple cider. On the other hand, UVC also destroyed d_4-furan in fructose solution. When fresh apple ciders were UV-treated to achieve the 5-log reduction of *E. coli*, as required by the U.S. FDA, less than 1 ppb furan was found. A significant amount of furan could be accumulated if apple cider was overtreated. Overall, our results suggest that little furan is induced in apple cider if UVC processing is used for the purpose of apple cider pasteurization.

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