Efficacy of Ultraviolet Light for Reducing *Escherichia coli* O157:H7 in Unpasteurized Apple Cider

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ABSTRACT

This study examined the efficacy of UV light for reducing *Escherichia coli* O157:H7 in unpasteurized cider. Cider containing a mixture of acid-resistant *E. coli* O157:H7 (6.3 log CFU/ml) was treated using a thin-film UV disinfection unit at 254 nm. Dosages ranged from 9,402 to 61,005 μW·s/cm². Treatment significantly reduced *E. coli* O157:H7 (P = 0.0001). Mean reduction for all treated samples was 3.81 log CFU/ml. Reduction was also affected by the level of background microflora in cider. Results indicate that UV light is effective for reducing this pathogen in cider. However, with the dosages used in this experiment, additional reduction measures are necessary to achieve the required 5-log reduction.

*Escherichia coli* O157:H7 was first identified as a foodborne pathogen in 1982 and is now acknowledged as a significant cause of foodborne illness (10). Recent outbreaks of *E. coli* O157:H7 infections involving contaminated deer jerky (16), mayonnaise (11), salami (5), and yogurt (22) have demonstrated the ability of this organism to survive in foods previously considered safe because of acidity, low water activity, or refrigeration. Apple cider or juice is also a novel vehicle for outbreaks of *E. coli* O157: H7 infections, and recently much attention has been focused on this problem.

An early outbreak of hemorrhagic uremic syndrome associated with apple juice or cider occurred in Canada before the recognition of *E. coli* O157:H7 as a foodborne pathogen; however, the responsible agent was not identified, possibly due to delays between sampling and analysis (28). Outbreaks of hemorrhagic uremic syndrome in Massachusetts in 1991 (3) and Connecticut in 1996 (7) attributed to *E. coli* O157:H7 were associated with drinking contaminated apple cider. In a 1996 outbreak in the Pacific Northwest, Odwalla brand unpasteurized apple juice and juice mixtures contaminated with *E. coli* O157:H7 were implicated, prompting a nationwide recall (6).

While apple juice is usually pasteurized, cider producers have relied upon the product’s inherent acidity, refrigeration, and chemical preservatives for preservation (9). However, numerous studies have demonstrated the ability of *E. coli* O157:H7 to survive in cider despite its low pH and regardless of the use of refrigeration or preservatives. Miller and Kaspar (21) reported survival of *E. coli* O157: H7 for at least 24 h in cider adjusted to a pH of 2 and enhanced survival at 4°C when compared to survival at 25°C, especially at lower pH. Survival was not affected by the use of preservatives. Likewise, Zhao et al. (36) reported longer survival times at 8°C than at 25°C with and without preservatives.

Many believe that pasteurization is the best means of eliminating *E. coli* O157:H7 from apple cider. Some larger juice processors have already begun pasteurizing (1). However, pasteurization may be cost prohibitive for smaller operations as costs tend to increase sharply as production capacity and number of days per year of processing decrease (18). Pasteurization may also affect the sensory characteristics responsible for the appeal of fresh cider (24).

The Food and Drug Administration (FDA) has proposed a regulation requiring fruit and vegetable juice processors to implement hazard analysis and critical control point programs. Part of this regulation includes requirements for processors of unpasteurized juices to adapt their processes to achieve a 5-log reduction of pathogens in the finished product (12). A technology that may be a more affordable means of achieving this reduction and prove less detrimental to sensory characteristics than pasteurization is the use of UV radiation.

The bactericidal action of UV is due to the destruction of nucleic acids, with the greatest effect at wavelengths between 250 and 260 nm (23). The sensitivity of bacteria to UV varies with species and also among different strains of the same species (4). The dosage necessary to cause a reduction of 99.9% in *E. coli* is comparable to that required for many other vegetative bacteria (8).

UV has been used for years for water sterilization, showing effectiveness against a wide variety of microorganisms (2, 35). It has also been used successfully on beef (15, 29), fish (14), and poultry (30, 33) to control bacteria and increase shelf life while causing little effect on food quality.

Little research exists on the use of UV to control pathogens in apple cider. UV has been used successfully to increase the shelf life of refrigerated cider without affecting flavor (32). Harrington and Hills (13) reported total micro-
bial reductions of from $6.6 \times 10^5$ to $1.4 \times 10^3$ CFU/ml in cider after exposure to UV for 54 s. Treated cider showed no signs of fermentation, spoilage, or changes in flavor for up to 35 days when stored at 2.2°C.

Despite these findings, UV irradiation has not been used commercially due to the problems presented by suspended solids in cider (9). The presence of small amounts of particulates in a liquid can greatly reduce UV penetration (2, 26). Kissinger and Willits (17) successfully eliminated microorganisms in flowing maple sap using UV radiation; however, the dissolved solids content of sap is considerably lower (1 to 4%) than that of apple juice (9.8 to 16.9%) (20). For UV radiation to be effective in liquids of such high UV absorptivity, the liquid must be subjected to UV in the form of a thin film (9). In this manner, UV absorption by the liquid is low and bacteria are more likely to be subjected to lethal doses (25). The inactivation of E. coli by UV light in solutions of high UV absorptivities using a thin film apparatus was described by Shama (25). The surviving fractions of E. coli present following treatment of water (UV absorptivity of 0.18) and humic acid solutions (UV absorptivity of 4.0) were $1.88 \times 10^{-5}$ and $1.84 \times 10^{-4}$, respectively.

The goal of this investigation was to determine the efficacy of UV light for the reduction of E. coli O157:H7 in apple cider. The effects of UV dosage and the initial level of background microflora in cider on inactivation were examined.

**MATERIALS AND METHODS**

**Test organism and culture maintenance.** Five acid-resistant E. coli O157:H7 strains 380-94, 933, C7927 (human isolate from cider outbreak), E0019, and E09 were obtained from the University of Nebraska, Lincoln culture collection. Stock cultures were maintained on tryptone soy agar (TSA; Difco Laboratories, Detroit, Mich.) at 4°C and grown in tryptone soy broth (Difco) at 35°C. Preliminary research determined that the level of inoculum obtained in tryptone soy broth for the five individual cultures ranged from 8.0 to 9.1 CFU/ml. Each culture was subjected to two successive transfers by loop inocula to 10 ml tryptone soy broth. A third transfer of 1 ml was made into 100 ml tryptone soy broth acidified to pH 5 with 1 N HCl for acid adaptation (19). Incubations were for 18 to 24 h at 35°C. The 100-mL cultures were combined in equal volumes to create a five-strain mixture.

**Inoculation and analysis of cider.** Apple cider was obtained from local producers in Virginia and stored at 4°C or −20°C until use. Frozen cider was thawed for analysis by placing at 4°C. Prior to inoculation, the level of background microflora in test cider was determined by spread plating serial dilutions (0.1% peptone) (Difco) on sorbitol MacConkey agar (SMAC; Difco) for E. coli O157:H7; TSA for aerobic organisms; and yeast and mold agar (Difco) supplemented with 0.01% chloramphenicol (Unipath Oxoid, Ogdensburg, N.Y.) for yeasts and molds. SMAC and TSA plates were incubated for 24 h at 35°C. Yeast and mold agar supplemented with 0.01% chloramphenicol plates were incubated for 48 h at 25°C. In addition, the pH and sugar content (°Brix) of the test cider were determined using a pH meter (Fisher Scientific, Pittsburgh, Pa.) and a Brix hydrometer (Fisher).

Cider samples with volumes ranging from 3 to 5 liters were transferred to sterile 5-liter flasks and inoculated with the five-strain mixture (3.5 ml/liter) to achieve an inoculum level of approximately $1.0 \times 10^6$ CFU E. coli O157:H7 per ml. Prior to UV treatment, inoculated cider was tested to determine the actual level of inoculum. Serial dilutions were spread plated on SMAC and TSA supplemented with 1% pyruvic acid (Fisher) (TSAP). TSAP was used to check for sublethally injured E. coli O157:H7. Plates were incubated for 24 h at 35°C. Typical sorbitol-negative colonies on SMAC and typical colonies on TSAP were enumerated with a Quebec colony counter. Questionable colonies present on TSAP were verified using biochemical and serological tests. Routine verification of isolates was conducted using Micro-ID’s (Remel, Lenexa, Kans.), and E. coli O157 Latex agglutination test kit (Unipath Oxoid).

**UV treatment.** Inoculated cider was pumped through a thin film UV disinfection unit, model CIDER-10uv (Ideal Horizons, Poultney, Vt.) using an air-operated diaphragm pump (Graco, Inc., Minneapolis, Minn.) or a peristaltic pump (Cole-Parmer Instrument Co., Vernon Hills, Ill.) depending on the desired flow rate. The UV disinfection unit operates at a peak radiation of 254 nm with a maximum intensity of approximately 107.366 μW/cm². Because fouling of the quartz sleeves that enclose the lamps tends to increase, and the efficiency of the lamps themselves tends to decrease over time, the applied intensity is somewhat less than maximum. The intensity of the unit at rated by the manufacturer is actually the end of lamp life intensity, which is 64,420 μW/cm² or 60% of the theoretical maximum. This is also the figure used in this research. The unit incorporates 10 individual UV chambers that are connected in series. UV dosage was varied by adjusting the flow rate of cider through the UV disinfection unit.

Cider flow rates (liters per minute, LPM) were measured with a flow meter (Fisher & Porter Co., Warminster, Pa.) attached to the outlet tubing on the UV disinfection unit. Following UV treatment, cider was collected in a sterile 2-liter flask. Recovered samples were immediately analyzed as detailed above for inoculated cider.

Prior to and following each treatment session, the UV unit and tubing were cleaned, sanitized, and rinsed. Cleaning and rinsing were accomplished by pumping hot water through the unit. A 500-ppm hypochlorite solution was used for sanitizing. Following the rinse step, the UV lamps were turned on for 10 min. Holding and collection vessels for cider were cleaned and sterilized before use.

**Experimental design and statistical analysis.** The experiment was run a total of 25 times at flow rates ranging from 0.999 LPM to 6.4837 LPM. This corresponds to maximum and minimum UV dosages of 61,005 and 9,402 μW-s/cm², respectively. Microbial counts (CFU/ml) were determined in duplicate for each replication. Counts were converted to log values and differences between untreated and UV-treated cider for each replication were calculated as log reduction factors (LRF; log CFU/ml).

Data were analyzed by regression analysis using the Statistical Analysis System (SAS Institute, Cary, N.C.) to determine significant differences among treatments.

**RESULTS AND DISCUSSION**

E. coli O157:H7 was not detected in any of the test ciders prior to inoculation. Background populations of aerobic mesophilic bacteria in test ciders ranged from undetectable (<1) to 4.4 with a mean of 2.2 log CFU/ml. Background populations of yeasts and molds ranged from <1 to 4.7 with a mean value of 3.4 log CFU/ml. The pH and sugar content did not vary greatly among the ciders used for testing. The pH of un inoculated test ciders ranged from...
TABLE 1. The effect of UV dosage on the reduction of E. coli O157:H7 in apple cider containing the following pretreatment levels of yeasts and molds

<table>
<thead>
<tr>
<th>Average UV dose (µW-s/cm²)</th>
<th>Log reduction factor (log CFU/ml)</th>
<th>Background yeasts and molds (log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61,005 (n = 2)</td>
<td>5.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>29,076 (n = 3)</td>
<td>3.6</td>
<td>4.7</td>
</tr>
<tr>
<td>18,641 (n = 1)</td>
<td>3.0</td>
<td>4.7</td>
</tr>
<tr>
<td>14,713 (n = 4)</td>
<td>3.0</td>
<td>4.2</td>
</tr>
<tr>
<td>12,100 (n = 2)</td>
<td>2.9</td>
<td>3.8</td>
</tr>
<tr>
<td>10,288 (n = 7)</td>
<td>3.1</td>
<td>4.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average UV dosage for all samples processed within a range of <2,000 µW-s/cm².

<sup>b</sup> Difference in microbial counts on sorbitol MacConkey agar between untreated and UV-treated cider.

<sup>c</sup> Values are means for all samples processed within each UV dosage range.

3.6 to 3.9 with a mean of 3.7. Sugar content ranged from 12.0 to 12.3 with a mean of 12.2 Brix.

Populations of E. coli O157:H7 in inoculated cider prior to UV treatment as enumerated on SMAC ranged from 5.3 to 6.5 with a mean value of 6.0 log CFU/ml. Using TSAP for the recovery medium, populations ranged from 5.7 to 6.8 with a mean of 6.3 log CFU/ml. The somewhat higher recovery on TSAP suggests that a portion of the cells were injured, possibly due to the pH of the cider or the presence of organic acids.

UV treatment significantly reduced E. coli O157:H7 in apple cider (P ≤ 0.0001). The mean LRF for all UV-treated samples was 3.8 and 3.6 log CFU/ml for SMAC and TSAP, respectively. This corresponds to a kill rate of >99.9%. In comparison, Harrington and Hills (13) reported an 88% reduction in total microbial counts for cider pumped through a UV irradiation unit at a rate of 1.449 LPM.

The lower reduction for TSAP may indicate apparent sublethal injury, possibly due to UV exposure. However, the difference in reduction between SMAC and TSAP was not statistically significant. In addition, because injured cells were recovered both before and after UV treatment, injury cannot be attributed solely to UV. Because the results did not differ significantly between media, only the results from SMAC will be presented.

The relationship between UV dosage, the pretreatment level of background yeasts and molds in cider, and reduction of E. coli O157:H7 is shown in Table 1. The values reported are means for samples processed within a range of 2,000 µW-s/cm². The UV dosages reported are averages of all dosages within a particular range. Because yeast and mold levels are typically higher in unpasteurized cider (9), data for samples with background yeast and mold populations of <2.0 log CFU/ml are not included in Table 1 with the following exception. Both samples processed at the highest UV dosage had yeast and mold levels of 1.0 log CFU/ml or less, and data for these samples are included for the sake of comparison. Yeast and mold levels are used here instead of aerobic bacteria because yeast and mold populations were generally higher than that of aerobic mesophilic bacteria. In addition, yeast cells are larger than bacteria and thus require lower numbers to cause turbidity (17).

Because the level of background microflora was not controlled in the experimental design, i.e., samples were randomly assigned to a treatment group, it was impossible to process samples with the same level at all UV dosages. However, the mean level of yeasts and molds varies by less than 1 log CFU/ml except for the top row in Table 1. As expected, the largest reduction resulted when samples with very low levels of background microflora were processed at the highest UV dosage.

In general, the LRF increases with an increase in UV dosage. This relationship is depicted graphically in Figure 1. However, the effect of UV dosage on reduction was not statistically significant. In addition, the level of background yeasts and molds in cider appears to have some effect. In fact, regression analysis found that the pretreatment levels of yeasts and molds in cider significantly affected reduction (P ≤ 0.03). Although not shown in Table 1, LRFs of 5 log CFU/ml or greater were only obtained when the combined level of background microflora was approximately 3 log CFU/ml or less, regardless of UV dosage.

Background microbial populations in liquids along with particulates and organic matter have been associated with low transmissivity of UV light (26). These findings are also consistent with those of Kissinger and Willits (17) who reported that UV reduction of microorganisms in maple sap was less effective in the presence of high levels of yeast.

The relationship between LRFs for UV-treated cider and pretreatment levels of background yeasts and molds was determined by linear regression analysis. The correlation coefficient was 0.44, suggesting that at least some of the variation in LRFs may be due to variances in yeast and mold levels. Higher levels of yeasts and molds in cider prior to treatment corresponded with lower LRFs.

As mentioned previously, the best reduction was obtained when cider with very low initial levels of yeasts and molds (1 log CFU/ml) was subjected to the highest UV dosage tested (61,005 µW-s/cm²). E. coli O157:H7 was reduced by an average of 5.4 log CFU/ml in such instances. This would meet the FDA requirement for a 5-log reduction. However, levels of background microflora, especially yeasts, in cider are typically much greater than 1 log CFU/ml (9). In addition, with the disinfection unit used for this research, a dosage this high was only achieved when cider was processed at a flow rate of approximately 1 liter per minute. A flow rate this slow is probably impractical for use in a commercial setting.

The mean level of E. coli O157:H7 in cider prior to UV treatment of approximately 6 log CFU/ml would appear to represent extreme levels of contamination. However, this level of contamination was necessary to conduct these studies. In comparison, the mean coliform count in 59 cider samples from 12 different producers was 2.74 log CFU/ml (27). For the same samples, the mean level of heterotrophic bacteria was 4.64. The highest coliform counts were for samples that had been produced using dropped apples (27).
In another study involving samples from 15 facilities, the average aciduric population of unpasteurized juice, including aciduric yeasts and molds, was 5 log CFU/ml (31).

With the equipment used in this study, the FDA requirement for a 5-log reduction of E. coli O157:H7 in unpasteurized cider would not likely be met using UV light alone. However, it could be met by using a combination of methods, including UV. Achieving such a reduction solely through the use of UV light would only be possible for cider with very low levels of background microflora processed at extremely slow rates. However, modifications to the unit to increase the intensity of UV irradiation as well as the maximal flow rate may make it possible to achieve greater reduction at faster processing speeds. Reduction will vary with the equipment used for UV disinfection as well as the nature of the liquid being treated. For this reason, it would be wise to investigate the effectiveness of UV for use in a particular situation (2).

Some advantages to the use of UV for the treatment of foods include the lack of residual chemicals and radiation, no changes in physical characteristics, and low capital and maintenance costs (23). The results of this investigation suggest that the potential exists for the use of UV light to help assure the safety of unpasteurized apple cider. At the very least, UV treatment could be used in conjunction with other preventive measures such as good manufacturing practices and sanitizing treatments for apples, as part of an approved hazard analysis and critical control point plan. In other research conducted in this laboratory, treatment of apples with 5% acetic acid resulted in a mean reduction in E. coli O157:H7 of 3.1 log CFU/cm² (34). According to the FDA (12), processors of unpasteurized juices would be free to employ a combination of methods to achieve the required 5-log reduction.

More research is needed to identify the actual UV dosage necessary to achieve a 5-log reduction of E. coli O157:H7 in unpasteurized apple cider containing typical levels of background microflora. In addition, the effect of UV treatment on the sensory characteristics of cider should be addressed. Also, because UV reduction of E. coli O157:H7 appears to be affected by background microbial populations in cider, this matter should be considered when conducting future studies. Finally, once an optimal dosage has been identified, researchers should consider the phenomenon of photoregeneration in UV-treated cider.

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