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Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157:H7 in Apple Juice and Apple Cider, Using Pulsed Light Treatment

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ABSTRACT

The main objective of this work was to evaluate the effectiveness of pulsed light (PL) treatment for the inactivation of *Escherichia coli* in liquids with different levels of clarity. Nonpathogenic *E. coli* ATCC 25922 and pathogenic *E. coli* O157:H7 were used as challenge organisms. Butterfield's phosphate buffer (BPB), tryptic soy broth (TSB), apple juice, and apple cider were used as substrates. The inoculated liquids were placed in a thin layer (1.3 mm) into glass chambers (23 by 53 by 11 mm) and exposed to PL doses of up to 13.1 J/cm^2. PL treatments were performed in a Xenon RS-3000C PL unit, both in static mode and under turbulence. Survivors were determined by standard plate counting or the most-probable-number technique. For static treatments, reduction levels exceeding 8.5 log were obtained in BPB for all strains and reduction levels of about 3.5 log were obtained in TSB. For apple juice, inactivation levels of 2.66 $\pm$ 0.10 log were obtained for *E. coli* ATCC 25922 and 2.52 $\pm$ 0.19 log for *E. coli* O157:H7. In cider, inactivation levels of 2.32 $\pm$ 0.16 log and 3.22 $\pm$ 0.29 log were obtained for the nonpathogenic and pathogenic strains, respectively. Inactivation kinetics was characterized using the Weibull model. Turbulent treatments resulted in 5.76 $\pm$ 0.06 log reduction in cider and 7.15 $\pm$ 0.22 log reduction in juice, which satisfies the U.S. Food and Drug Administration requirement of 5-log reduction of *E. coli.* These results show promise for the use of PL for the effective reduction of *E. coli* in apple juice and cider.

Consumption of unpasteurized apple cider caused several outbreaks of *Escherichia coli* O157:H7 in recent years *(5, 7, 9)*. *E. coli* O157:H7, which primarily affects children, the elderly, and immunocompromised individuals, causes a wide range of health effects, from mild cases of diarrhea to hemolytic uremic syndrome *(7, 9)*. The most serious outbreak of *E. coli* O157:H7 linked to apple cider in the United States occurred in October 1996, when at least 70 people became sick and one child died after drinking unpasteurized apple juice *(7)*. One of the reasons for these outbreaks is the fact that *E. coli* O157:H7 has the ability to survive in cider despite low pH, as well as under refrigeration or even in the presence of preservatives *(12)*. For instance, *E. coli* O157:H7 can survive storage in apple cider (pH 3.6 to 4.0) for 10 to 31 days at 8°C or 2 to 8 days at 25°C *(13)*.

As a reaction to the increasing occurrence of food poisoning outbreaks linked to cider and juice, the U.S. Food and Drug Administration (FDA) adopted new regulations in 2001 requiring all processors to include at least one treatment step that achieves a minimum 5-log reduction in pertinent pathogens or to provide a warning label on the bottle *(22)*. Pasteurization is still perceived as the securest way to obtain a 5-log reduction of pathogens, but many cider and juice processors, especially the small producers, are reluctant to use pasteurization due to high costs and decrease in enzymes, color, and flavor as a result of pasteurization *(6)*. According to the FDA, the safety assurance step does not necessarily have to be a heat treatment. One of the FDA-approved methods to treat juice and cider is UV light treatment. UV was shown to lead to 5- to 6-log reductions of *E. coli* in apple cider at UV doses of $\geq$6,500 μW-s/cm^2 *(2, 16)*, with much less significant effects on cider quality than those occurring with heat treatment *(8)*.

Pulsed light (PL) treatment has emerged in recent years as a feasible alternative to thermal treatment for inactivation of pathogenic and spoilage microorganisms. Its use has been approved by the FDA for the decontamination of food and food surfaces with the restriction that a Xenon flashlamp has to be used as a light source, with pulse durations of $<$2 ms and the cumulative treatment not exceeding 12 J/cm^2 *(21)*. PL treatment involves the use of intense and short-duration (1 μs to 0.1 s) pulses of broad-spectrum light of wavelength ranging from UV to near-infrared (200 to 1,100 nm). It has been suggested by some authors that PL treatment can achieve higher levels of microbial reduction than continuous UV treatment *(11)*, although this issue is still debated. The specific causes of cell death in PL treatment are not fully elucidated, but progress has been made in recent years in this area. A majority of the studies on PL indicate the significant contribution of the UV portion of the spectrum on microbial inactivation, with the lower-wavelength UV having a more pronounced microbicidal effect *(1, 18, 23, 25)*.

Previous work indicates that PL can be used against *E. coli* O157:H7. Roberts and Hope in 2003 *(17)* reported a 6.2-log CFU reduction of *E. coli* O157:H7 in phosphate

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buffer saline after 200 pulses of light, Sharma and Demirci *(19)* achieved a 4.89-log CFU reduction on alfalfa seeds after 270 pulses, and Ozer and Demirci *(15)* reported a 1.09-log CFU reduction on raw salmon filets after 180 pulses of light. For the last two studies, the reported fluence was 5.6 J/cm^2 at the strobe surface. The different levels of PL effectiveness in these studies can be attributed mostly to the influence of the treated substrate, since absorption, reflection, or scattering of light by the substrate can diminish the effectiveness of the treatment. The effect of surface properties on microbial inactivation on solid substrates by PL has been discussed in detail by Woodling and Moraru *(24)* and Uesugi et al. *(20).* Less information is available, however, regarding the effect of liquid substrates on microbial inactivation by PL.

The main objective of this work was to evaluate the effectiveness of PL treatment for the inactivation of *E. coli* in liquids with different levels of clarity, including apple juice and apple cider. The suitability of nonpathogenic *E. coli* ATCC 25922 to be used as a surrogate organism for *E. coli* O157:H7 in PL treatment was also investigated.

MATERIALS AND METHODS

Liquid substrates. Four different liquid substrates of different degrees of clarity were chosen: unpasteurized, unfiltered apple cider, apple cider clarified by cold microfiltration (which will be referred to as ''apple juice'' in this article), Trypticase soy broth (TSB), and Butterfield's phosphate buffer (BPB). BPB is a clear solution with light absorption characteristics similar to those of water, while TSB does have the visual appearance of a clear, yellow-brownish liquid.

The unpasteurized apple cider, which did not contain any preservatives or other additives, was obtained from the Cornell Dairy Store (Ithaca, NY) and stored at −20°C until use. Frozen cider was thawed in a refrigerator at 4°C and then autoclaved for 30 min at 121°C prior to the PL treatments. Clarified cider was obtained from the same batch of raw cider by cold microfiltration using a Tami Isoflux ceramic membrane (GEA Filtration, Hudson, WI) of 0.45-μm pore size, with 23 channels of 3.5-mm hydraulic diameter. The process parameters for microfiltration were as follows: crossflow velocity, 7 m/s; transmembrane pressure, 0.7 bar; temperature, 6°C.

The pH and sugar content (in degrees Brix) of the cider and juice were determined using a pH meter (Fisher Scientific, Pittsburgh, PA) and a Digital Fiberoptic Refractometer (Misco, Cleveland, OH), respectively.

Optical properties of the substrates. The absorbance spectrum of the liquid substrates in the range of 200 to 1,100 nm was determined using a high-resolution fiber optic spectrometer, HR2000+CG-UV-NIR (Ocean Optics; Dunedin, FL; software, OOIBASE32), at an optical resolution of 0.035 nm.

Challenge organisms and inoculum preparation. The challenge microorganisms used in this work were pathogenic and nonpathogenic strains of *E. coli* in stationary growth stage. The nonpathogenic *E. coli* ATCC 25922 was identified as a potential surrogate organism for *E. coli* O157:H7, based on this organism's response to continuous UV treatment *(2, 16). E. coli* ATCC 25922 (clinical isolate from the American Type Culture Collection) was obtained from the Food Microbiology Laboratory at the New York State Agriculture Experiment Station (Geneva, NY) and kept on Trypticase soy agar (TSA; Becton Dickinson, Sparks, MD). Prior to the PL treatments, a single isolated colony was transferred into TSB (Becton Dickinson) and incubated for 20 ± 2 h at 35 ± 2°C. An aliquot of 1 ml was transferred into the working substrate immediately before the PL treatments, resulting in a population of approximately 10^9 CFU/ml.

As pathogenic inoculum, a three-strain cocktail of *E. coli* O157:H7 was used. The cocktail consisted of a mixture of *E. coli* O157:H7 ATCC 43889 (isolated from a patient with hemolytic uremic syndrome from North Carolina), *E. coli* O157:H7 ATCC 43895 (isolated from raw hamburger meat implicated in a hemorrhagic colitis outbreak), and *E. coli* O157:H7 strain 933 (meat isolate). All three strains were obtained from the Food Microbiology Laboratory at the New York State Agriculture Experiment Station. It has to be noted that *E. coli* O157:H7 strain ATCC 43895 is listed as a 933 strain by the American Type Culture Collection, but it is different from the other 933 strain used in this study. The cultures were kept in a fashion similar to that of the nonpathogenic strain. Prior to PL treatments, a single isolated colony of each *E. coli* O157:H7 strain was transferred into TSB and incubated for 20 ± 2 h at 35 ± 2°C, and 0.333 ml of each culture was transferred into the respective liquid substrate immediately before the PL treatment, resulting in a population of approximately 10^9 CFU/ml.

PL treatments. Equal volumes (1 ml) of the inoculated liquid substrates were placed in sterile and transparent Lab-Tek II Chamber Slide 1 well slides (Nagle Nunc International, Naperville, IL) with chamber dimensions of 23.2 by 53.3 by 11.0 mm. The bottom of the chamber was made of glass, while the walls were made of polystyrene. The thickness of the liquid layer in the chamber slide was 1.3 mm.

The PL treatments were performed using an RS-3000C SteriPulse System (Xenon Corporation, Woburn, MA). The system consists of a controller unit and a treatment chamber that houses a Xenon flash lamp. For the static treatments, each inoculated chamber was centered individually on an adjustable stainless steel shelf in the PL unit at known distances beneath the lamp and treated with up to 12 pulses of light, at a frequency of 3 pulses per s and a pulse width of 360 μs. The treatment levels were 0 (control), 1, 3, 6, 9, and 12 pulses.

In order to generate turbulence in the liquid substrates during the PL treatment, an MS 3 basic orbital shaker (IKA Works, Wilmington, NC) with a variable speed, ranging from 0 to 3,000 rpm, was placed in the center of the stainless steel shelf in the PL unit. The Lab-Tek II Chamber Slides were secured in place on the universal attachment of the shaker, at 55.9 mm beneath the Xenon lamp.

All PL treatments were performed in duplicate, with the exception of the turbulent treatments performed for the nonpathogenic *E. coli,* which were performed in triplicate.

PL fluence measurements. The dose of the PL treatments (fluence), expressed in joules per square centimeter, was quantified for all treatments. Fluence measurements were taken using a pyroelectric head (PE25BBH) with a Nova II display (Ophir Optronics Inc., Wilmington, MA). The pyroelectric head was placed centrally on an adjustable stainless steel shelf in the PL unit at given distances beneath the Xenon lamp. A stainless steel aperture cover was placed over the head and completely covered the top surface of the head except for a 1-cm^2 circular opening that exposed the detector's surface. The opening was centered on the detector's surface. On the Nova II display, the pulse width setting was 1.0 ms and the wavelength setting was <0.3 nm. Fluence measurements were performed in triplicate. Pauses of at least 60

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s between measurements were allowed in order to prevent overheating of the pyroelectric head.

Temperature measurements. To quantify the heating of the liquid substrates that occurred as a result of the PL treatment, the temperature of the substrate was measured before and after the PL treatment. Temperature measurements were performed in triplicate using a noncontact, type K infrared thermometer (Fisher Scientific). Samples were prepared as described above (1 ml in Lab-Tek II Chamber Slide 1 well slides), placed 50.8 mm underneath the lamp, and treated with up to 12 pulses of light. Immediately after the PL treatment, the chambers were taken out of the PL unit, placed on a white surface, and tilted to an angle of about 30°. The head of the thermometer was placed at a distance of about 15 cm from the liquid surface, and the temperature was recorded. The time elapsed between the cessation of the PL treatment and the temperature measurement was about 3 s.

Enumeration of survivors. Following the PL treatment, the treated inoculum (1 ml) was transferred into 7 ml of TSB, and the glass chamber was rinsed twice with 1 ml of TSB, adding the rinse TSB to the treated inoculum. The recovery liquid (10 ml) was serially diluted in BPB, after which 100 μl was spread plated onto TSA and incubated for 48 ± 2 h at 35 ± 2°C. Survivors were counted using the standard plate counting method. When plate counts fell below 25 CFU, estimation of survivors was performed using the most-probable-number technique (MPN) *(20)*. The treated liquid (10 ml) was transferred into 100 ml of TSB in the following distribution: 3 tubes with 10 ml, 3 tubes with 1 ml, and 3 tubes with 0.1 ml. The TSB tubes then were incubated at 35 ± 2°C for 48 ± 2 h. Turbidity was used to presumptively identify positive samples. The inoculum (N_0) and survivor counts (N) were expressed in log CFU and log MPN, respectively. All standard plate counting and MPN analyses were performed in duplicate.

The level of microbial reduction was calculated as $\log(N/N_0)$, and inactivation curves were built by plotting reduction against fluence (F).

Kinetic modeling of inactivation curves. The nonlinear Weibull model was used to describe inactivation kinetics *(20)*. For microbial inactivation by PL, the Weibull function takes the following form:

$$\frac{N}{N_0} = e^{-(F/\alpha)^\beta}, \quad \text{or}$$

$$\ln\left(\frac{N}{N_0}\right) = -\left(\frac{F}{\alpha}\right)^\beta, \quad \text{or}$$

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{2.303}\left(\frac{F}{\alpha}\right)^\beta \qquad (1)$$

where N/N_0 represents the ratio of survivors after treatment over the initial number of organisms, α is the scale parameter, β is the shape factor, which describes the shape of the survivor curve, and F is the treatment intensity, or fluence (in joules per square centimeter). The Weibull parameters α and β were obtained by linearizing equation 2, using Microsoft Excel (Microsoft Inc.).

Statistical analysis. For each treatment, the mean and standard deviation for the survivor ratios were calculated. Analysis of variance and Tukey's honestly significant difference statistical tests were used to determine significant differences at P values of <0.05, using Minitab 14.2 (Minitab Inc.).

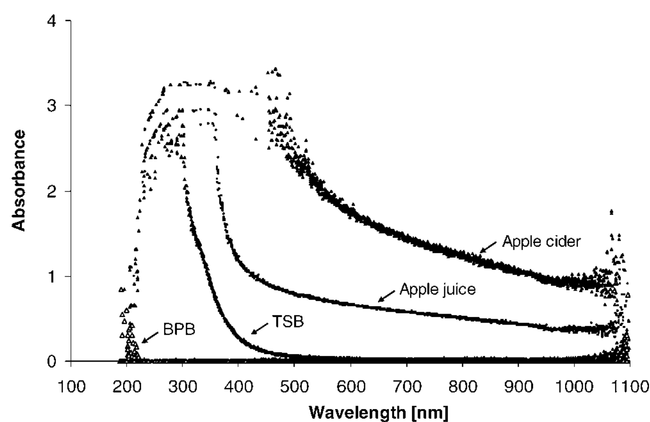

FIGURE 1. *Absorbance spectra in the UV–visible light–near-infrared range for the liquid substrates.*

RESULTS AND DISCUSSION

Substrate characterization. As light absorption by the substrate diminishes the amount of light available to the microbial cells, it was important to quantify the absorption properties of the treated substrates. The absorbance spectra of the four liquid substrates were measured, and the results are shown in Figure 1. Since the UV range is the portion of the broad-spectrum light that is critical for microbial inactivation *(25)*, absorption in the UV range is the most important for the outcome of PL treatment. While BPB did not show significant absorbance of UV light, the three other substrates absorbed fairly high amounts of light in this range. Both apple juice and apple cider showed high absorbance around λ of 254 nm, the region that is often credited as having the maximum bactericidal effect *(3)*. The absorbance spectrum of TSB was very similar to the one of apple juice, with the difference that TSB absorbed less light at λ of $>$300 nm.

Another important substrate characteristic was the pH. The pH of the treated cider and juice was about 4.0, while TSB and the BPB solution had pH values of 7.4 and 6.8, respectively. In preliminary experiments, *E. coli* ATCC 25922 was inoculated in apple cider overnight, which resulted in an initial inoculum level of 5.93 ± 0.40 log CFU/ml, about 3 log below the cell concentration that was obtained when the inoculum was prepared in buffer. This reduction showed that this *E. coli* strain was sensitive to low pH. Therefore, in order to minimize the effect of the substrate pH on the inactivation results, in all subsequent treatments the substrates were inoculated immediately prior to the PL treatments.

The solids concentration of the substrates, quantified by the degree Brix, is not expected to have an influence on inactivation. A study by Murakami et al. *(14)* showed that Brix levels did not affect inactivation rates of *E. coli* during continuous UV treatment. In this work, the Brix values of the substrates were as follows: BPB, 0.5 °Bx; TSB, 3.25 °Bx; apple juice, 11.25 °Bx; and apple cider, 11.25 °Bx.

Effect of substrate-light interaction on inactivation of *E. coli*. The four substrates were inoculated with 9.18 ± 0.13 log CFU of *E. coli* ATCC 25922 per ml and ex-

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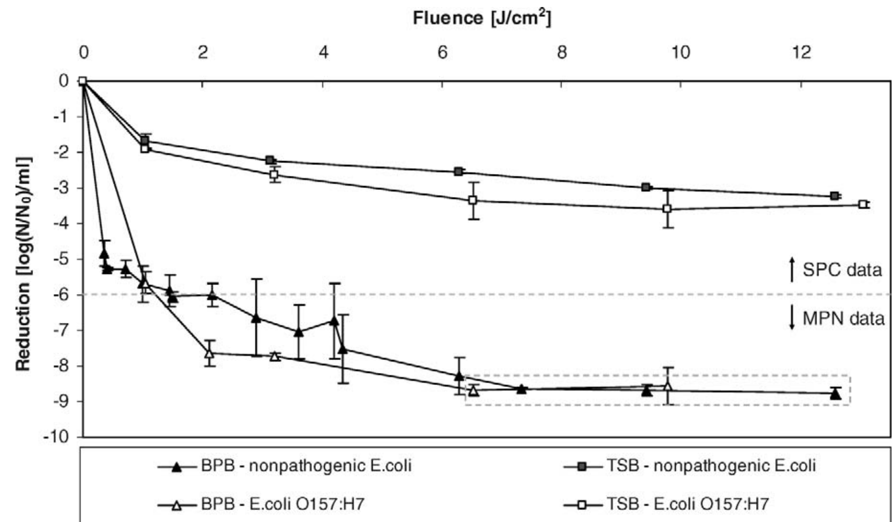

FIGURE 2. *PL inactivation of* E. coli *ATCC 25922 and pathogenic* E. coli *O157:H7 three-strain cocktail in BPB and TSB under static conditions. The dotted line represents the boundary between the data obtained using the standard plate counting technique and those obtained by the MPN technique. Data in the dotted rectangle were obtained at the detection limit of the MPN method.*

posed to up to 12 pulses of PL. PL inactivation levels for *E. coli* ATCC 25922 in BPB were significantly higher than in cider, juice, or TSB (Figs. 2 and 3). In the case of PL treatment of BPB at fluence levels higher than 2.0 J/cm^2, the MPN technique was used to estimate the number of survivors. The dotted line in Figure 2 represents the boundary between the data obtained using the standard plate counting technique and the data obtained by the MPN technique.

At a fluence (F) of 0.4 J/cm^2 the reduction of *E. coli* ATCC 25922 was 5.29 ± 0.04 log CFU, and the maximum recorded reduction was 8.75 ± 0.16 log CFU, at F = 12.6 J/cm^2. The near-total reduction of *E. coli* in BPB at high fluence levels challenged even the sensitivity of the MPN method. In Figure 2, the data points inside the dotted rectangle represent inactivation levels recorded at the sensitivity limit of the MPN method, i.e., survivor counts of $<$0.48 log CFU.

The PL treatment of TSB, apple juice, and apple cider resulted in comparable inactivation levels. For apple cider and juice, after treatment with 1 pulse (F = 1.1 J/cm^2) inactivation levels of *E. coli* ATCC 25922 of 1.49 ± 0.15 and 1.62 ± 0.09 log CFU/ml, respectively, were obtained. After 12 pulses (F = 12.6 J/cm^2) the reductions were 2.32 ± 0.16 and 2.66 ± 0.10 log CFU/ml for apple cider and juice, respectively (Fig. 3). Lower inactivation levels for *E. coli* ATCC 25922 in apple cider than in BPB, TSB, and clear juice were expected, due to the presence of suspended particles in the cider, which could cause shielding of microbial cells from the incident light, and also due to its significant UV absorption. Surprisingly, the reduction levels in apple cider and clear apple juice were very similar, and no statistical difference between the inactivation levels, at the same PL dose, was noticed between the two substrates ($P > 0.05$). The PL treatment of *E. coli* ATCC 25922 in TSB resulted in reduction levels of 1.69 ± 0.21 log CFU/ml for 1 pulse and 3.24 ± 0.05 log CFU/ml for 12 pulses, respectively. Inactivation levels of *E. coli* ATCC 25922 in TSB were not statistically different from those obtained in the juice and cider treatments at 1.0 J/cm^2 and 3.2 J/cm^2, respectively ($P > 0.05$).

These results suggest that the level of inactivation was limited mostly by the absorption of light in the UV range. Both apple juice and cider showed high absorbance of light in the range of wavelengths from 200 to 400 nm, while TSB absorbs smaller amounts of light at wavelengths of

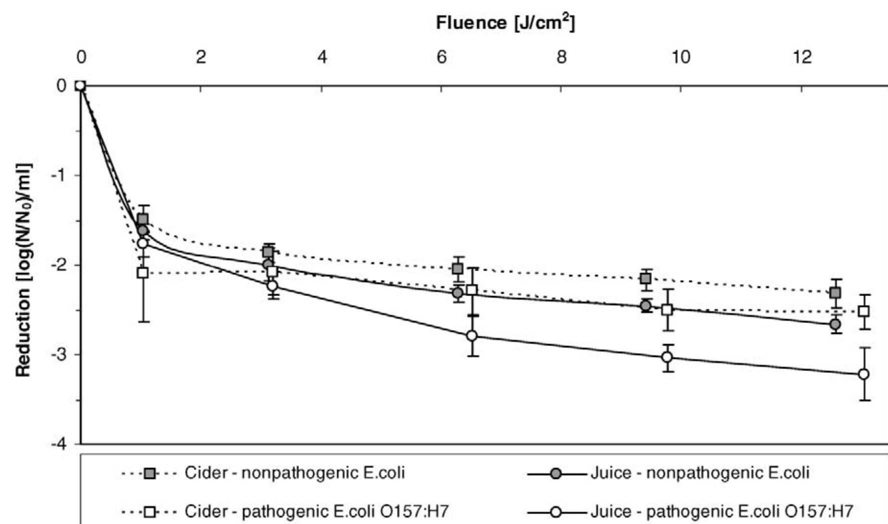

FIGURE 3. *PL inactivation of* E. coli *ATCC 25922 and pathogenic* E. coli *O157:H7 three-strain cocktail in apple juice and apple cider under static conditions.*

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>300 nm, which leads to higher inactivation levels of *E. coli* ATCC 25922 in this substrate than in juice and cider. These observations are consistent with the findings of Koutchma and Parisi *(10),* who found that the UV absorbance of different caramel model solutions of apple juice and cider affected the efficiency of continuous UV light treatment.

Validation of *E. coli* ATCC 25922 as a surrogate organism for *E. coli* O157:H7. The efficacy of any antimicrobial technology is typically tested in the laboratory by using the most relevant pathogens for a particular application as challenge organisms. Before its commercial application, the efficacy of the process must be validated using surrogate microorganisms. To date, no surrogate identification and validation has been reported for the pathogenic *E. coli* O157:H7 treated with PL. To establish whether the nonpathogenic *E. coli* ATCC 25922 is an appropriate surrogate for *E. coli* O157:H7, PL treatments of the four liquid substrates were also performed on a three-strain cocktail of *E. coli* O157:H7. The three strains were chosen since they have been used before as challenge organisms in UV treatments *(2).* The inactivation curves for the pathogenic and nonpathogenic strains are shown in Figure 2 (for BPB and TSB) and Figure 3 (for apple juice and apple cider).

For all substrates, inactivation curves for the pathogenic and nonpathogenic strains had nearly identical shapes and similar levels of inactivation in all substrates. For BPB, although the initial part of the inactivation curve looks slightly different for the pathogenic strain and for the nonpathogenic strain, this could be due to the larger number of data points available for the nonpathogenic strain at F values of <1.0 J/cm^2. With the exception of a difference at F of ~2 J/cm^2, where inactivation for the pathogenic cocktail was significantly higher than for the nonpathogenic strain in BPB, PL treatment seemed to have the same effectiveness for the two situations. The slightly lower inactivation levels for the nonpathogenic strain than for the pathogenic cocktail (albeit in most cases not statistically significant at P values of <0.05) actually represent a favorable result, since this would allow a security factor when predicting inactivation of pathogenic *E. coli* O157:H7 based on the PL inactivation behavior of the nonpathogenic *E. coli* ATCC 25922.

These findings suggest that *E. coli* ATCC 25922 can be used as a surrogate for the pathogenic *E. coli* O157:H7 in PL treatment. This bears a very important practical significance since, based on the results of this study, *E. coli* ATCC 25922 can be used to validate PL processes in a commercial setting where inactivation of *E. coli* O157:H7 is desired.

Inactivation kinetics of *E. coli* in PL treatment. When characterizing microbial inactivation kinetics, the first-order model is widely accepted. However, since PL inactivation curves are clearly nonlinear, as shown both in this study and in previous reports *(20, 24, 25),* the use of first-order kinetics is unsuitable for PL inactivation. According to Uesugi et al. *(20),* the nonlinear Weibull model can be used to quantitatively describe microbial inactivation

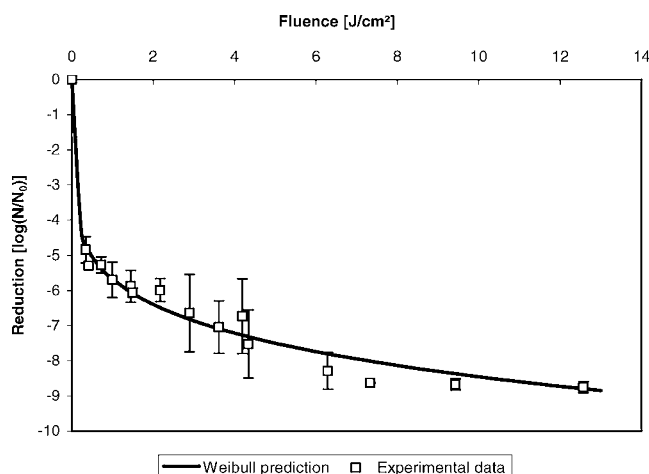

FIGURE 4. *Experimental and Weibull calculated inactivation data for* E. coli *ATCC 25922 in BPB.*

by PL. For PL treatment, the Weibull function takes the following form:

$$\log(S) = -\alpha \cdot F^{\beta} \quad (2)$$

where $S = (N/N_0)$, the ratio of survivors after the treatment over the initial number of organisms, α is the scale parameter, β is the shape factor, which describes the shape of the survivor curve, and F is the PL treatment dose, or fluence.

In order to obtain the Weibull parameters, equation 2 was linearized, the logarithm of log(S) was plotted against the logarithm of fluence, and the Weibull parameters were obtained from the regression equation $y = \beta \cdot x + n$, where β is the Weibull shape factor and n is the logarithm of α. For *E. coli* ATCC 25922 in BPB, the Weibull equation took the following form:

$$\log(N/N_0) = -5.70 \cdot F^{0.17} \quad (3)$$

As seen in Figure 4, which depicts both the experimental and calculated inactivation data for inactivation of *E. coli* ATCC 25922 in BPB, the Weibull model was able to represent accurately the survivor ratios in PL inactivation. Similarly, a very good fit of the Weibull calculated inactivation with the experimental data was found for all substrates. The calculated shape (β) and scale (α) parameters for the four liquid substrates for the PL inactivation of nonpathogenic and pathogenic *E. coli* are shown in Table 1. The α and β values for the nonpathogenic and pathogenic strains are comparable, and the small differences lie within the biological variability. Another observation is that for all substrates very similar values of the shape parameter β were obtained, but the values of α, which reflects the magnitude of PL inactivation, were quite different.

Increasing the effectiveness of PL treatment for inactivation of *E. coli* by using turbulence. Despite the relatively low reduction levels (around 2 log) of both pathogenic and nonpathogenic *E. coli* in apple juice and apple cider, the potential of this treatment is much higher, as demonstrated by the near-complete inactivation of the same strains in clear BPB (Fig. 2). It is clear that the interaction of light with the apple juice and apple cider limited the

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TABLE 1. *Weibull parameters for the PL inactivation of* E. coli *ATCC 25922 (nonpathogenic) and* E. coli *O157:H7 three-strain cocktail (pathogenic) in liquid substrates*

Substrate	*E. coli* strain	No. of data points	Shape parameter β	Scale parameter α	Goodness of fit (R^2)
Cider	Nonpathogenic	5	0.17	1.49	1.00
	Pathogenic	5	0.08	2.01	0.99
Juice	Nonpathogenic	5	0.20	1.60	1.00
	Pathogenic	5	0.25	1.73	1.00
TSB	Nonpathogenic	5	0.25	1.66	1.00
	Pathogenic	5	0.26	1.94	0.98
BPB	Nonpathogenic	15	0.17	5.70	0.98
	Pathogenic	5	0.18	6.10	0.98

effectiveness of PL treatment. While absorption of UV light is determined by the chemical composition of these substrates and thus cannot be modified, it is possible to counteract, at least to some extent, the shading effects caused by the particulates present in the nonclear liquid substrates (TSB, apple juice, and apple cider), by performing the PL treatment under turbulence. The use of turbulence is very relevant for a potential commercial application of PL for processing of fruit juices or other liquid foods, since such treatments would be most likely performed in a flow-through system, in which turbulence can be precisely controlled. In the absence of a flow-through PL unit, a system that allowed the generation of controlled turbulence was devised by placing the glass chambers onto the top plate of a variable-speed orbital shaker inside the PL unit. PL treatments of 1 to 12 pulses were carried out at two different velocities of the shaker, which allowed the creation of low turbulence (500 rpm) and high turbulence (3,000 rpm) during the PL treatment. In order to minimize the change of the light path inside the PL unit due to the presence of the shaker, all of the shaker parts were completely wrapped in aluminum foil. Fluence measurements were performed for the modified experimental setup in order to allow the accurate quantification of treatment dose.

Inactivation curves for PL inactivation of *E. coli* ATCC 25922 at different levels of turbulence are shown in Figures 5 and 6. Turbulence had virtually no effect on the inactivation level for the PL treatment in clear BPB, as in this case the inactivation curves for the static treatment and for the low-turbulence treatment overlapped (data not shown). This suggests that the full inactivation potential of PL in this clear substrate has been reached even in the static setting, because there was no cell shielding by the substrate. While in a thicker layer of liquid turbulence might have benefits even for the treatment of clear liquids, a thin-layer treatment can be performed either in static conditions or, in the case of a flow-through system, under laminar flow conditions.

For the other three liquid substrates, turbulence had a clear effect on inactivation, and PL effectiveness increased significantly with increasing turbulence. The treatments carried out under high turbulence for TSB and apple juice (Figure 6) at fluence levels higher than 5.5 J/cm^2 (6 pulses) and 3.8 J/cm^2 (4 pulses), respectively, the MPN technique had to be used to estimate the number of survivors. In apple juice a maximum reduction of 7.29 ± 0.73 log CFU was achieved with high turbulence (at F = 8.8 J/cm^2), compared with 4.46 ± 0.39 and 2.66 ± 0.10 log CFU for the treatment with low turbulence (F = 8.8 J/cm^2) and for the static treatment (F = 12.6 J/cm^2), respectively. For the high-turbulence treatment, inactivation levels of *E. coli* ATCC 25922 in apple cider of up to 5.49 ± 0.06 log CFU were achieved, which was about 2.4 log CFU higher than for the low-turbulence treatment and about 3.2 log CFU higher than for the static treatment. The experimental data confirmed that turbulence can significantly enhance the ef-

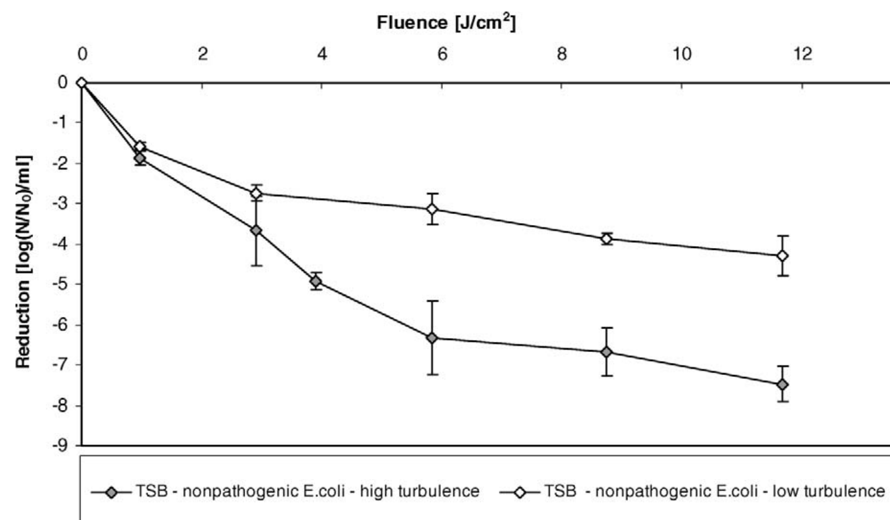

FIGURE 5. *Inactivation of* E. coli *ATCC 25922 in TSB at different turbulence levels. Data points at* F > *5.5 J/cm^2 for the high turbulence treatments are based on MPN estimates.*

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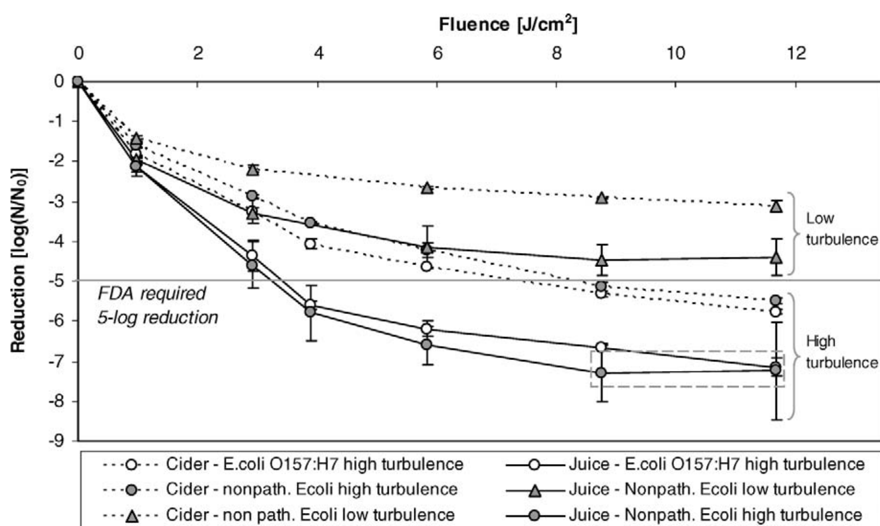

FIGURE 6. *PL inactivation of nonpathogenic* E. coli *ATCC 25922 and pathogenic* E. coli *O157:H7 three-strain cocktail in apple juice and apple cider under turbulence. Data at* F $>$ *5.5 J/cm*^{2} *for high turbulence are based on MPN estimates. Data in the dotted triangle were obtained at the sensitivity limit of the MPN method.*

fectiveness of PL treatment, presumably by maximizing exposure of microbial cells to the incident light. Turbulence could also disintegrate clusters or clumps of microbial cells, which can also lead to increased microbial inactivation. A very important observation is that both for apple juice and for apple cider the FDA-required 5-log reduction of *E. coli* was achieved under high turbulence at PL doses below 12 J/cm^2, which is the FDA-imposed limit for PL treatment.

The effect of turbulence on microbial PL inactivation in apple juice and cider has also been tested for the pathogenic three-strain cocktail (dotted curves in Fig. 6). Maximum inactivation levels of 7.15 $\pm$ 0.22 log CFU for apple juice and 5.76 $\pm$ 0.06 log CFU for apple cider have been reached under the high-turbulence treatment. The inactivation levels for the pathogenic and nonpathogenic strains in apple juice were not statistically different from each other ($P > 0.05$), and for apple cider only slight differences occurred. This further demonstrates that *E. coli* ATCC 25922 can be used as a surrogate organism for *E. coli* O157:H7 in PL treatment.

As in the static treatments, the inactivation curves for turbulent treatments were also nonlinear, and for BPB, TSB, and apple juice a clear plateau was observed at high fluence levels. For TSB and apple juice, the plateau was more pronounced for the high-turbulence treatment than for low turbulence. In apple cider turbulent treatments a clear plateau was not observed, particularly for the high-turbulence treatment. This suggests that the full inactivation potential of *E. coli* in apple cider may not have been reached and that higher turbulence levels than those used in the present study might lead to higher inactivation levels. This observation is very important for the potential commercial applications of PL treatment in apple cider processing, because it might lead to inactivation levels higher than the 5.5-log results obtained in the current study.

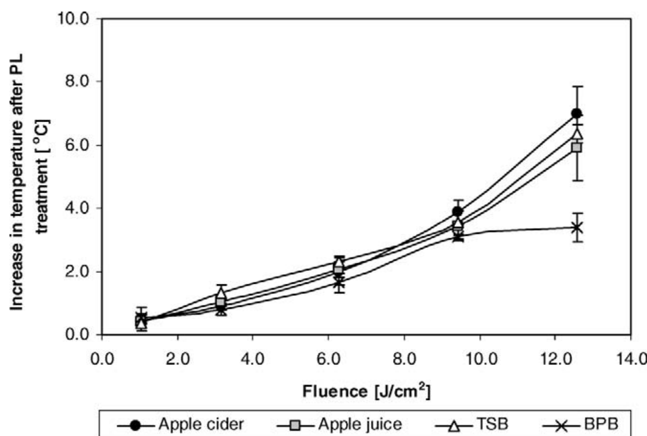

FIGURE 7. *Measured temperature increase in the treated fluids as a function of PL dose.*

Heating effects as a result of the PL treatment. The increase in temperature as a result of PL treatment was tested for all substrates, and the results are shown in Figure 7. The final temperature of the treated liquids increased with the PL dose. The measured increase in temperature seemed also to be substrate dependent, with the smallest heating effects observed in the clearest substrate (BPB) and the highest heating effects occurring in the most turbid and absorbent substrate (apple cider). It must be noted, though, that the largest increase in temperature was about 7°C, corresponding to a measured temperature of about 34°C (in apple cider). This level of heating is modest, and it is not expected to result in any significant chemical or sensory changes of the treated liquids, although sensory and chemical analysis should be performed to confirm this hypothesis. One also has to take into account that the way in which the temperature was measured may underestimate slightly the actual temperature increase during the treatment.

Overall, the results of this study indicate that absorption of light, particularly in the UV region, and shielding of microorganisms by suspended matter are significant limiting factors in PL treatment of microorganisms in liquid substrates. This was expected and consistent with studies that have investigated the effectiveness of continuous UV for microbial inactivation in liquids *(4)*.

It was also demonstrated that nonpathogenic *E. coli* ATCC 25922 can be used as a surrogate for the pathogenic *E. coli* O157:H7 in PL treatment. Turbulence can drastically enhance the level of microbial kill in PL treatment, facili-

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tating the achievement of the required 5-log reduction of *E. coli* O157:H7 in apple juice and cider, without heating. The current results indicate much promise for the use of PL treatment for the effective reduction of *E. coli* in a turbulent, thin-layer flow configuration. One of the big advantages of PL over static UV treatment is the fact that the energy is delivered in a very short time. The levels of inactivation reported in the present study were achieved in less than 4 s, which highly recommends this process for in-line applications, with minimal modifications of existing processing lines.

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