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Antimicrobial Efficacy of UV Radiation on *Escherichia coli* O157:H7 (EDL 933) in Fruit Juices of Different Absorptivities

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ABSTRACT

The efficacy of UV light for inactivating *E. coli* (ATCC 25922) and *E. coli* O157:H7 (EDL 933) was examined in fruit juices (orange, apple, and multifruit) with different absorptivities under several operating conditions (liquid film thickness and agitation rate). The juices were inoculated with two bacterial concentrations (10^5 and 10^7 CFU/ml) and were treated using a UV desinfection unit at 254 nm; UV doses ranged from 0 to 6 J/cm^2. The effect of the culture medium, tryptone soy agar (TSA) and sorbitol MacConkey agar (SMAC), on the recovery of *E. coli* strains exposed to UV radiation was also analyzed. The most suitable culture medium for recovery of *E. coli* strains in juices exposed to UV radiation was TSA. Values of *D* (radiation dose [joules per square centimeter] necessary to decrease the microbial population by 90%) obtained in all juices assessed were higher in TSA than in SMAC. In the juices analyzed, stirring of the medium exposed to UV radiation and reducing liquid film thickness (to 0.7 mm) produced the highest bactericidal effect. A linear relationship was found between the *D*-values obtained and the absorptivity coefficients for all the juices. The higher the absorbance of the medium, the greater the values of *D* required to inactivate *E. coli* strains by UV radiation. An equation was developed to describe the relationship of the fraction of energy absorbed by the system (absorbed energy factor [AEF]), the thickness of the film exposed to UV radiation, and the absorptivity coefficient of the juices. A linear relationship was found between *D* and AEF in the different juices tested.

Enterohemorrhagic *Escherichia coli* O157:H7, a cause of hemorrhagic colitis and hemolytic uremic syndrome, has been largely associated with undercooked ground beef *(22)* and was first identified as a human foodborne pathogen in 1982 *(7)*. *E. coli* O157:H7 infections have been associated with a variety of foods, including raw milk *(3),* raw vegetables *(5),* pork *(15),* lamb *(16),* poultry and contaminated water *(29),* and highly acidic foods such as apple cider *(3),* mayonnaise *(4),* yogurt, and salami. These outbreaks have illustrated the ability of this organism to survive in foods previously considered safe for their acidity, low water activity, or refrigerated condition *(30)*.

Apple, orange, and other acidic fruit juices are also novel vehicles for *E. coli* O157:H7 infections, and in the last years much attention has been focused on this problem because these products also have been implicated in outbreaks of gastroenteritis. An early outbreak of hemolytic uremic syndrome associated with apple juice and cider occurred in Canada before *E. coli* O157:H7 had been recognized as a foodborne pathogen. The responsible agent in this outbreak was not identified, possibly because of delays between sampling and analysis *(27)*. In fall 1991, an outbreak of hemorrhagic colitis associated with apple cider occurred in Massachusetts *(31)*. 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 *(30)*.

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Several methods have been developed for isolation of *E. coli* O157:H7 from food and clinical samples based on the fact that this enteric bacterium is unable to ferment sorbitol or produce β-glucuronidase. Sorbitol MacConkey agar (SMAC) is routinely used as a selective differential plating medium *(1)*.

The Center for Food Safety and Applied Nutrition found in its preliminary study that unpasteurized juices accounted for 76% of juice contamination cases reported between 1993 and 1996. Approximately 16,000 to 48,000 illnesses per year can be attributed to these juices *(9)*.

The Food and Drug Administration (FDA) issued a final rule to increase the safety of fruits and vegetable juice and juice products. According to this regulation, juice processors must use hazard analysis and critical control point principles for processing and must achieve a 5-log (100,000-fold) reduction in the number of the most resistant pathogens in their finished products compared with concentrations likely to be present in untreated juices. The rule, effective on January 2002, states that approved alternatives to pasteurization can be used to achieve microbial reduction *(10)*.

Nonthermal technologies have been proposed to achieve this level of reduction in fruit and vegetable juices while preserving heat-sensitive flavor and aroma components. Treatment with UV radiation could be an attractive alternative technology to thermal pasteurization. Some authors believe that pasteurization is the best mean of eliminating *E. coli* O157:H7 from apple cider. One currently recommended pasteurization treatment for apple juice is

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heating to 71.1°C for 6 s *(14)*. However, pasteurization may be too expensive for small-scale operations where specific processing costs tend to increase sharply as production capacity decreases *(20)*. The use of UV light in foodstuffs has many advantages over chemical disinfectants because it leaves no residues and has a relatively low cost.

Inactivation of microorganisms by UV radiation occurs because of damage to nucleic acids, with greater effects at wavelengths between 250 and 260 nm *(19)*. Nucleic acid damage is the result of absorption of the radiation by the DNA and the dimerization of thymine bases. These thymine dimers distort the double helix DNA conformation and interfere with normal DNA replication *(12)*; thus, these thymine dimers on bacterial DNA strands are lethal *(17)*.

UV radiation has been used successfully on solid and liquid foods such as beef *(28),* fish *(13),* and apple cider *(23)* and for water disinfection *(26)*. A shortcoming of the use of UV radiation in liquid foods is the presence of small amounts of suspended solid particles, which can greatly reduce penetration of UV radiation. The dissolved solids in apple juice range from 9.8 to 16.9% *(18)*.

Food characteristics must be taken into account when selecting UV radiation doses required to eliminate microorganisms. One of these characteristics is the absorptivity, defined as the proportionality constant between food absorbance (at 254 nm) and its concentration.

The objectives of the present work were (i) to determinate the efficacy of UV light for inactivating a pathogenic *E. coli* O157:H7 strain and a nonpathogenic *E. coli* strain in fruit juices with different absorptivity values under several conditions of film thickness and agitation rates, (ii) to analyze the effect of the culture medium on the recovery of *E. coli* from fruit juice previously exposed to UV light, and (iii) to assess the effect of the initial number of inoculated *E. coli* cells on the survival curves and to mathematically model the results obtained.

MATERIALS AND METHODS

Commercial pasteurized fruit juices (orange, apple, and multifruit) were purchased at a local supermarket. These types of juices were selected because they do not contain microbial preservatives. The pH of the juices was determined using an electrode (model 50215, Hach, Loveland, Colo.) on a pH meter (model EC30, Hach). The refraction index and sugar content (°Brix) was determined by an Abbe-type refractometer (Bellingham and Stanley, Kent, UK).

Two strains of *E. coli*, ATCC 25922 (nonpathogenic) and O157:H7 EDL 933 (hamburger isolate, kindly provided by Dr. Marta Rivas, National Reference Laboratory, ANLIS, Dr. Carlos G. Malbrán, Buenos Aires, Argentina), were used in the current study. Stock cultures were maintained on tryptone soy agar (TSA; Difco, Becton Dickinson, Sparks, Md.) at 4°C and grown in tryptone soy broth (TSB; Difco, Becton Dickinson) at 37°C for 18 h. A preliminary study indicated that the inoculum concentration obtained in TSB for both individual cultures ranged from 9.0 to 9.5 log CFU/ml.

Each culture was subjected to two successive transfers by loop (10 μl) to 10 ml of TSB and incubated at 37°C for 6 h. A third transfer of 1 ml was made into 100 ml of TSB. Incubation was performed at 37°C for 18 to 24 h.

Inoculation and analysis of fruit juice. Background aerobic microbial populations were estimated by the pour plate method on TSA incubated for 37°C at 24 h. Mold and yeast populations were enumerated via surface plating on yeast extract–glucose–chloranphenicol agar (Merck KGaA, Darmstadt, Germany) incubated at 25°C for 5 days. Presence of generic *E. coli* was determined according to AOAC method 46016 *(2)*. *E. coli* O157:H7 was identified on SMAC (Difco, Becton Dickinson) after incubation at 37°C for 24 h.

Samples (99 ml) of each juice were placed individually in sterile bottles and inoculated with 1 ml of both *E. coli* ATCC 25922 and *E. coli* O157:H7 EDL 933 to achieve two different inoculum concentrations of approximately 10^5 CFU/ml (low [L] concentration) or 10^7 CFU/ml (high [H] concentration).

Prior to UV treatment, inoculated fruit juices were tested to determine the actual *E. coli* populations. Serial dilutions (1:10) were performed with sterile 0.1% peptone water, followed by spread plating of selected dilutions in duplicate on TSA and SMAC plates. Typical colonies were counted after incubation at 37°C for 24 h, and the log CFU per milliliter values were calculated. TSA was used to check for sublethally injured *E. coli.*

Determination of juice UV absorptivity. The effect of suspended solids on the inactivation of both *E. coli* strains exposed to the UV radiation was evaluated by utilizing three juices with different absortivity coefficients. To obtain this coefficient, different dilutions in sterile water of orange, apple, and multifruit juices were prepared, and their absorbances at 254 nm were determined in 1-cm light path quartz cuvettes with a spectrophotometer (DU 650, Beckman Coulter, Palo Alto, Calif.). The regression curve slope obtained by plotting absorbance versus sample concentration was considered the absortivity coefficient.

UV treatment. A low-vapor-pressure mercury source of germicide UV light at 254 nm (UV Lux 30W/G30 T8, Philips, Eindhoven, The Netherlands) was used for irradiating the samples. Each sample was placed in a temperature-controlled chamber set to 20°C. UV light intensities at 254 nm were measured using a UV radiometer (model VLX-3W CE, Vilbert Lourmat, Marne La Vallee, France); an average value of 3.00 ± 0.5 mW/cm^2 was applied in each test. UV radiation doses ranged between 0 and 6 J/cm^2 and were obtained by multiplying the measured UV intensity and the time (in seconds) of exposure.

To analyze the effect of film thickness when exposing the sample to UV radiation, 20 and 5 ml of each inoculated juice were placed in sterile petri dishes separately to obtain layers 2.8 mm and 0.7 mm thick, respectively. The effect of stirring was studied by placing the samples on an orbital shaker regulated between 0 and 220 rpm. The distance between the sample and the UV lamp was 15 cm. Depending on operating conditions, exposure times to UV radiation ranged from 0 to 30 min, from several seconds for thin stirred layers of juice with low absorptivity to several minutes for thick stagnant layers of food with high absorptivity.

Enumeration of surviving *E. coli.* Surviving populations of each *E. coli* strain following exposure to UV radiation were enumerated by plating on both culture media (TSA and SMAC). Appropriate serial dilutions (1:10) were made in 9 ml of sterile 0.1% peptone water. Inoculation, irradiation, and sampling generally took less than 1 h. All plates were incubated for 24 h at 37°C. Colonies were counted, and the results were expressed as log CFU per milliliter; *E. coli* counts were performed in duplicate.

Inactivation kinetics. Mathematical models allow the effect of different factors on microbial survival to be analyzed. One of

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the tested models was the ''single hit–single target'' model, according to the following expression:

$$(N/N_0) = e^{-kF} \quad (1)$$

where k is the inactivation rate coefficient, F is the UV dose (J/cm^2), N is the microbial concentration (CFU per milliliter) at any time t, and N_0 is the initial microbial concentration (CFU per milliliter). This kinetic equation implies that cells are rendered inactive when subjected to a single harmful event, i.e., a single ''hit.'' The number of these hits experienced by a single cell will be directly proportional to the UV dose. Single hit–single target inactivation events are identical to first order kinetics.

Another model employed to describe UV inactivation was the multitarget survival function:

$$(N/N_0) = 1 - (1 - e^{-kF})^n \quad (2)$$

where k is the inactivation rate coefficient, F is the UV dose, n is the number of targets, N is the microbial concentration at any time t, and N_0 is the initial microbial concentration. If the value of n is set equal to 1.0, then the single hit–single target kinetics is obtained (equation 1). Although first order kinetics is the simplest mathematical form of a kinetic expression applicable to cell inactivation, it cannot be applied to all situations. In multitarget kinetics, the cell is visualized as containing multiple targets, all of which must be struck by UV photons for cell death to occur. Multitarget survival functions manifest themselves by plateaus or ''shoulders'' at low UV doses *(11)*.

Peleg and Cole *(21)* also suggested the Weibull distribution model for describing bacterial survival curves as the cumulative form of a temporal distribution of lethal events. Therefore, each individual bacterium dies at a specific time. The population displays a spectrum of individual resistances against the detrimental factors. This model predicts

$$\log N/N_0 = Kt^{-m} \quad (3)$$

Equations were fitted to experimental data by linear and nonlinear regressions using Systat software (Systat, Evanston, Ill.).

Experimental design and statistical analysis. A factorial design was used: three juices were tested (apple, orange, and multifruit), two layer thicknesses (0.7 and 2.8 mm), two stirring rates (0 and 220 rpm), two strains (*E. coli* ATCC 25922 and O157:H7 EDL 933), and two inoculum concentrations (L and H). UV doses ranged between 0 and 6.0 J/cm^2. Therefore, a total of 48 experimental conditions were tested for each UV dose (3 juices $\times$ 2 thicknesses $\times$ 2 stirring rates $\times$ 2 strains $\times$ 2 inocula concentrations). All tests were done in duplicate. Systat software was also used to determine significant differences among treatments based on an analysis of variance (ANOVA). Confidence intervals of each slope of the linear regressions also were determined.

RESULTS AND DISCUSSION

E. coli was not detected in any of the tested juices before inoculation. Background populations of aerobic mesophilic bacteria, yeast, and molds were not detectable in any of the analyzed fruit juices. Average pH values of the juices were 3.53 for orange, 3.47 for apple, and 3.76 for multifruit. Refraction index of all juices was 1.35. Sugar contents in the juices in °Brix were 12.52 for orange, 12.35 for apple, and 14.0 for multifruit.

The ANOVA yielded significant differences ($P < 0.05$) in the initial microbial populations obtained in both culture media. To compare the performance of each recovery medium (TSA and SMAC), two levels of initial microbial populations for each *E. coli* strain were tested.

Initial bacterial population (N_0, CFU/ml) in TSA of the pathogenic and the nonpathogenic *E. coli* in juices (control) ranged from $10^{4.88}$ to $10^{4.99}$ for the L inoculum and from $10^{6.55}$ to $10^{6.71}$ for the H inoculum. These values were lower in SMAC than in TSA, with initial counts ranging from $10^{4.32}$ to $10^{4.52}$ and $10^{5.94}$ to $10^{6.09}$ for L and H inocula, respectively. Because SMAC is a more selective medium for estimating surviving *E. coli* populations, these results suggest that a portion of the cells was injured possibly by the low juice pH or the presence of organic acids, leading to better growth in a nutritive culture medium (TSA) *(29)*.

Effect of initial inoculum population. Figure 1 shows surviving population curves expressed as log N/N_0 for *E. coli* O157:H7 EDL 933 in apple, orange, and multifruit juices that were inoculated with L and H initial microbial populations, exposed to UV light, and enumerated in TSA as the recovery medium. Juice films with thicknesses of 0.7 and 2.8 mm and kept under stirring or stagnant conditions were analyzed. Figure 2 shows result for *E. coli* ATCC 25922.

Similar trends were observed using SMAC as recovery medium (data not shown); however, bacterial populations were in all cases higher in TSA. Under all conditions tested (type of juice, layer thickness, and stirring rate), surviving populations expressed in dimensionless form as log N/N_0 were not significantly different for the two initial (L and H) bacterial concentrations.

Mathematical model. Among the three mathematical models tested, the first order kinetics yielded the closest agreement with data; the coefficients of determination (r^2) of the linear regressions ranged between 0.91 and 0.99. Slopes of the linear regressions (k) were determined, and the 95% confidence intervals were calculated for both strains and each treatment using TSA and SMAC as recovery media (Table 1). Microbial counts for all the tested conditions were significantly different ($P < 0.05$) in the two recovery media.

Values for D (the radiation dose necessary to decrease the microbial population by 90%) were calculated by taking the negative reciprocal of the slope corresponding to the linear regressions of the survival curves. Values obtained using TSA or SMAC as recovery media are shown in Tables 2 and 3, respectively. The significance of the differences between slopes was assessed by ANOVA. Based on the D-values, the bactericidal action of UV on both *E. coli* strains in juices were in order of effectiveness as follows: apple > orange > multifruit.

Effect of the culture medium on *E. coli* counts. D-values were between 1.2 and 1.8 times higher for cultures on TSA than for those on SMAC for all the juices studied, indicating that TSA allows for enumeration of sublethally damaged cells. Thus, TSA was considered the more suitable culture medium for *E. coli* strains in UV-exposed juices. The FDA recommends a 5D reduction for inactivation of *E. coli* O157:H7 in fruit juices when using alternatives to

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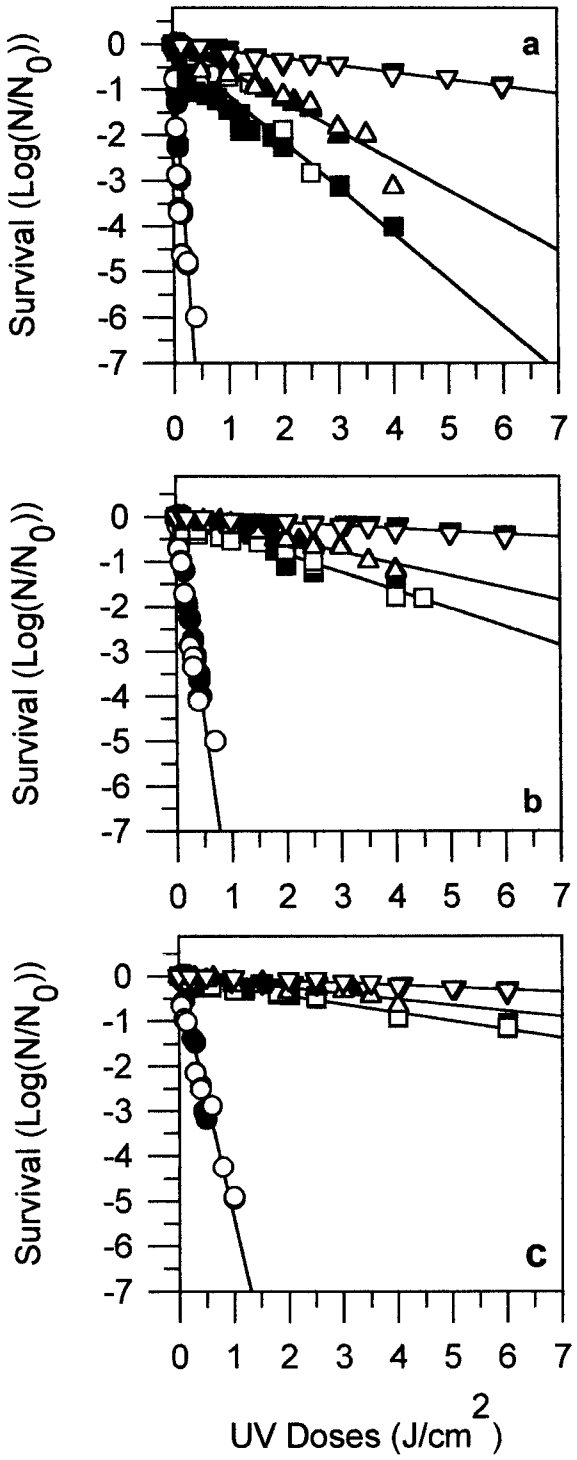

FIGURE 1. *UV inactivation (at 254 nm) of* E. coli *O157:H7 EDL 933 in apple (a), orange (b), and multifruit (c) juices at different film thicknesses and stirring conditions with TSA as recovery medium. ●, 0.7 mm, stirred (220 rpm); ■, 0.7 mm, stagnant (0 rpm); ▲, 2.8 mm, stirred; ▼, 2.8 mm, stagnant. Closed symbols indicate survival counts obtained with high inoculum concentration; open symbols indicate counts obtained with low inoculum concentration.*

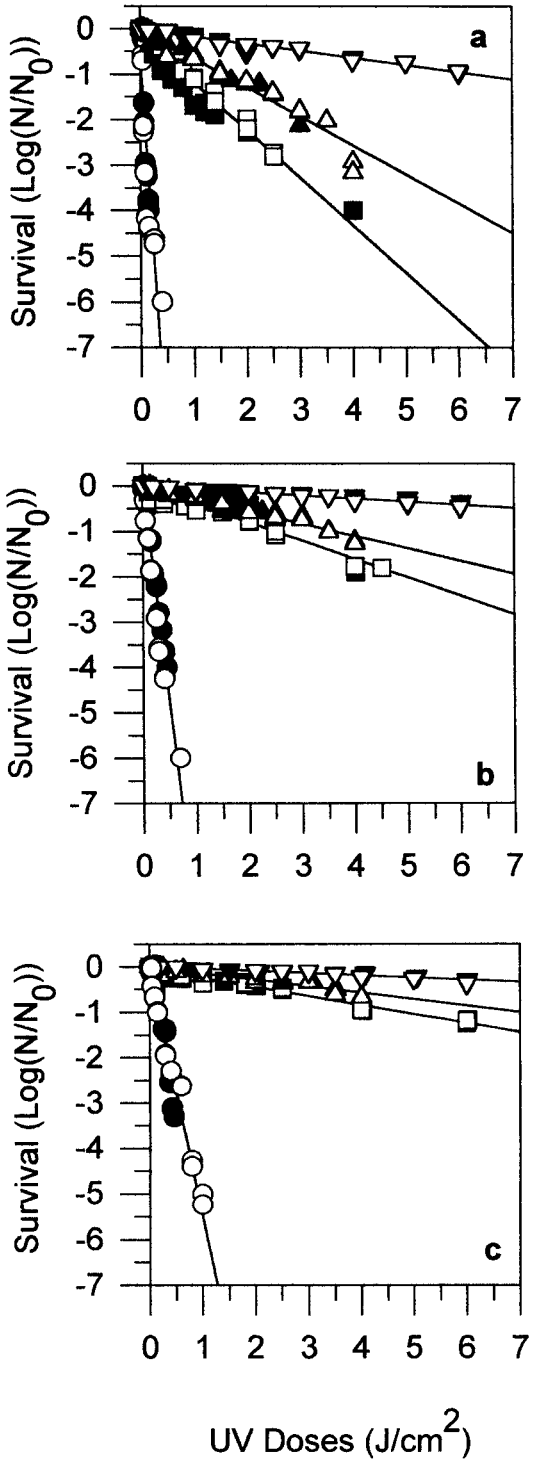

FIGURE 2. *UV inactivation (at 254 nm) of nonpathogenic* E. coli *ATCC 25922 in apple (a), orange (b), multifruit (c) juices at different film thicknesses and stirring conditions with TSA as recovery medium. ●, 0.7 mm, stirred (220 rpm); ■, 0.7 mm, stagnant (0 rpm); ▲, 2.8 mm, stirred; ▼, 2.8 mm, stagnant. Closed symbols indicate survival counts obtained with high inoculum concentration; open symbols indicate counts obtained with low inoculum concentration.*

pasteurization. Count errors can be made when SMAC is used as the culture medium for *E. coli* O157:H7; injured *E. coli* O157:H7 cells were reported to be unable to form colonies on SMAC plates. The difference in populations yielded by TSA and SMAC spread plates can be used to estimate the extent of cell injury *(24)*.

Other authors *(14, 30)* did not find significant *E. coli* O157:H7 count differences for inoculated apple cider plated

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TABLE 1. *First order kinetics coefficient (k) of the survival curves for* E. coli *ATCC 25922 and O157:H7 EDL 933 in apple, orange, and multifruit juices after UV treatments using TSA and SMAC as recovery media*

	Slope of the linear regression (k) with a liquid film thickness of[a]:							
	0.7 mm				2.8 mm			
	Stirred		Stagnant		Stirred		Stagnant	
Fruit juice	TSA	SMAC	TSA	SMAC	TSA	SMAC	TSA	SMAC
				***E. coli* ATCC 25922**				
Apple	−32.08 ± 1.48	−59.61 ± 3.16	−1.04 ± 0.02	−1.54 ± 0.05	−0.64 ± 0.01	−0.84 ± 0.01	−0.15 ± 0.003	−0.19 ± 0.005
Orange	−9.28 ± 0.22	−15.74 ± 0.25	−0.40 ± 0.01	−0.73 ± 0.05	−0.27 ± 0.005	−0.38 ± 0.004	−0.06 ± 0.001	−0.10 ± 0.005
Multifruit	−5.42 ± 0.14	−6.78 ± 0.26	−0.20 ± 0.003	−0.34 ± 0.01	−0.14 ± 0.004	−0.22 ± 0.003	−0.05 ± 0.001	−0.07 ± 0.002
				***E. coli* O157:H7 EDL 933**				
Apple	−30.85 ± 1.35	−61.29 ± 3.15	−1.01 ± 0.03	−1.69 ± 0.04	−0.66 ± 0.01	−0.81 ± 0.02	−0.15 ± 0.002	−0.19 ± 0.002
Orange	−9.05 ± 0.24	−14.70 ± 0.27	−0.40 ± 0.01	−0.76 ± 0.04	−0.27 ± 0.007	−0.35 ± 0.003	−0.06 ± 0.001	−0.12 ± 0.005
Multifruit	−5.26 ± 0.11	−6.12 ± 0.20	−0.19 ± 0.004	−0.36 ± 0.01	−0.13 ± 0.004	−0.22 ± 0.004	−0.05 ± 0.001	−0.07 ± 0.002

[a] 95% Confidence intervals of the first order kinetics coefficients were obtained as $k \pm ts(k)$, where k is the slope, t is the value obtained from Student's t test, with $n - 2$ degrees of freedom, and $s(k)$ is the standard deviation.

FIGURE 3. *Regression of absorbance at 254 nm versus juice concentration (milliliter of juice per milliliter of solution). The slope of the regression line is the absorptivity coefficient. a, apple juice; b, orange juice; c, multifruit juice.*

on TSA or SMAC. This contrasting behavior can be attributed to the use of *E. coli* strains preadapted for the acidic conditions of the medium, which may result in a lower degree of injury imparted by the juice. For 1-mm-thick apple juice films under stirring conditions, the UV radiation dose necessary to reach a 5*D* reduction when SMAC was used as the culture medium was 0.1 J/cm^2; however, when TSA was used, only a 3*D* reduction was achieved with this dose.

Effect of juice absorptivity coefficient on effectiveness of UV radiation. For both *E. coli* strains tested, the radiation dose required to attain a 5*D* reduction in stirred 0.7-mm-thick orange juice films was 0.55 J/cm^2; however, this dose resulted in only a 3-log decrease in multifruit juice under the same conditions. To explain the influence of the

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TABLE 2. D- *and* r²*-values for pathogenic and nonpathogenic* E. coli *in apple, orange, and multifruit juices with different values of film thickness under stagnant or stirred conditions after UV radiation treatment using TSA as recovery medium*

	Values for juices with a liquid film thickness of:							
	0.7 mm				2.8 mm			
	Stirred		Stagnant		Stirred		Stagnant	
Fruit juice	r^2	D^a	r^2	D	r^2	D	r^2	D
				***E. coli* ATCC 25922**				
Apple	0.91	0.03 (0.002)	0.95	0.96 (0.03)	0.96	1.56 (0.05)	0.97	6.67 (0.18)
Orange	0.95	0.11 (0.004)	0.93	2.50 (0.06)	0.95	3.70 (0.11)	0.96	16.67 (0.55)
Multifruit	0.95	0.18 (0.010)	0.97	5.00 (0.12)	0.94	7.14 (0.31)	0.94	20.00 (0.80)
				***E. coli* O157:H7 EDL 933**				
Apple	0.92	0.03 (0.002)	0.93	0.99 (0.04)	0.95	1.51 (0.04)	0.98	6.67 (0.13)
Orange	0.94	0.11 (0.004)	0.95	2.50 (0.06)	0.96	3.70 (0.14)	0.93	16.67 (0.55)
Multifruit	0.96	0.19 (0.006)	0.96	5.26 (0.17)	0.91	7.69 (0.35)	0.91	20.00 (0.80)

[a] *D*-values are given in joules per square centimeter with standard errors given in parentheses.

type of juice on *D*-values, the absorptivity of each juice was measured.

In the present work, absorptivity values were obtained for the different juices from the linear regression of absorbance at 254 nm versus concentration. The following absorptivities and coefficients of determination were obtained: apple juice, 0.0715 (r^2= 0.999); orange juice, 0.3528 (r^2 = 0.998); and multifruit juice, 0.7230 (r^2 = 0.999) (Fig. 3).

Figures 4 and 5 show the linear relationship between *D*-values and absorptivity coefficients. This relationship was not modified by the culture medium and the strain employed because there were no significant differences in the behavior of the pathogenic and the nonpathogenic strains of *E. coli* (Figs. 4 and 5). In liquid foods of high absorptivity, such as multifruit juice, higher UV doses are required to reach bactericidal effect; in consequence, lower radiation doses were required to inactivate *E. coli* strains in apple juice. Shama *(25)* found that UV action on *E. coli* strains was strongly influenced by the absorptivity of the medium

TABLE 3. D- *and* r²*-values for pathogenic and nonpathogenic* E. coli *in apple, orange, and multifruit juices with different values of film thickness under stagnant or stirred conditions after UV radiation treatment using SMAC as recovery medium*

	Values for juices with a liquid film thickness of:							
	0.7 mm				2.8 mm			
	Stirred		Stagnant		Stirred		Stagnant	
Fruit juice	r^2	D^a	r^2	D	r^2	D	r^2	D
				***E. coli* ATCC 25922**				
Apple	0.94	0.02 (0.001)	0.97	0.65 (0.03)	0.98	1.19 (0.03)	0.97	5.26 (0.22)
Orange	0.99	0.06 (0.001)	0.91	1.37 (0.15)	0.99	2.63 (0.04)	0.98	10.00 (0.40)
Multifruit	0.94	0.15 (0.008)	0.98	2.94 (0.09)	0.99	4.54 (0.10)	0.97	14.28 (0.61)
				***E. coli* O157:H7 EDL 933**				
Apple	0.94	0.02 (0.001)	0.98	0.59 (0.02)	0.98	1.23 (0.04)	0.98	5.26 (0.19)
Orange	0.99	0.07 (0.002)	0.92	1.31 (0.10)	0.99	2.85 (0.03)	0.94	8.33 (0.49)
Multifruit	0.94	0.16 (0.008)	0.98	2.78 (0.08)	0.99	4.54 (0.12)	0.96	14.28 (0.61)

[a] *D*-values are given in joules per square centimeter with standard errors given in parentheses.

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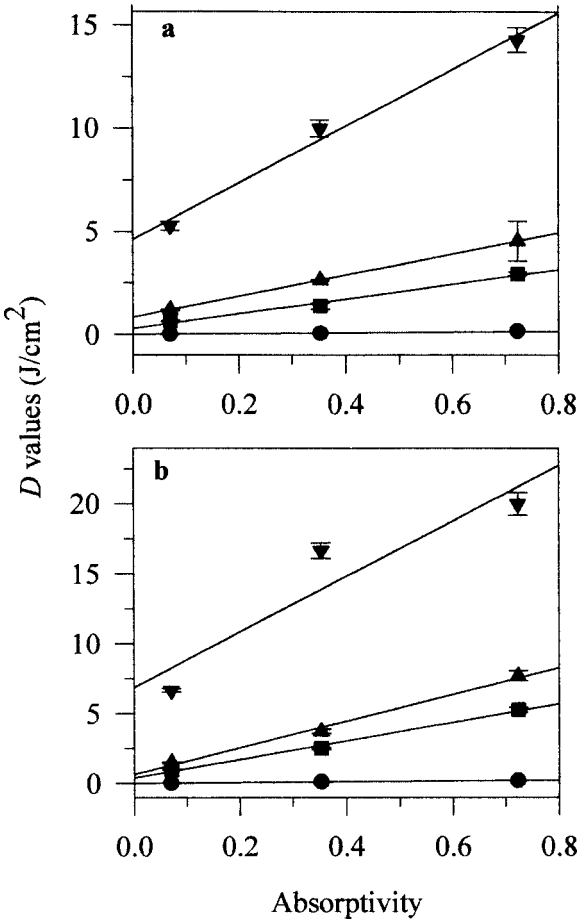

FIGURE 4. *Relationship between* D-*values and absorptivity coefficients for different film thicknesses and stirring conditions with TSA as recovery medium.* ●, *0.7 mm, stirred;* ■, *0.7 mm, stagnant;* ▲, *2.8 mm, stirred;* ▼, *2.8 mm, stagnant. (a)* E. coli *ATCC 25922. (b)* E. coli *O157:H7 EDL 933. Bars indicate standard deviation of the mean.*

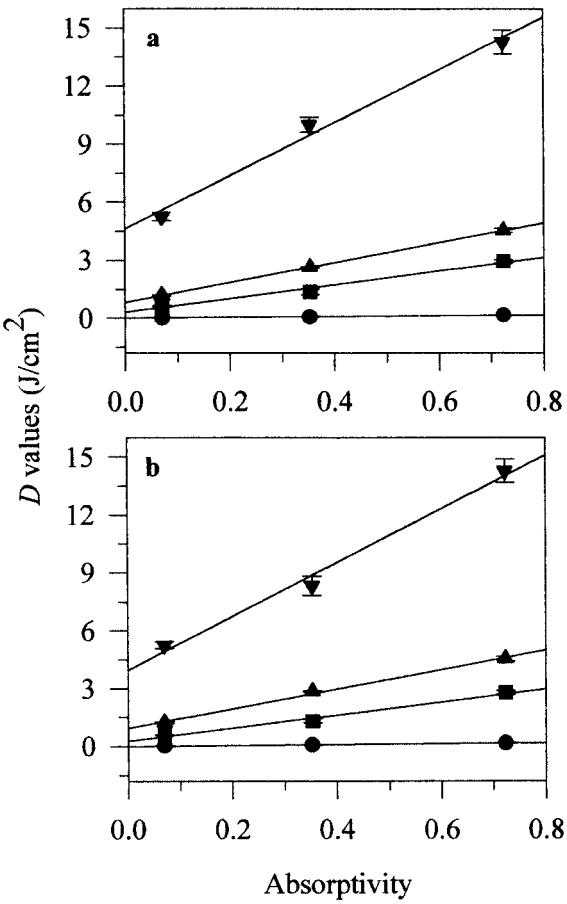

FIGURE 5. *Relationship between* D-*values and absorptivity coefficients for different film thicknesses and stirring conditions with SMAC as recovery medium.* ●, *0.7 mm, stirred;* ■, *0.7 mm, stagnant;* ▲, *2.8 mm, stirred;* ▼, *2.8 mm, stagnant. (a)* E. coli *ATCC 25922. (b)* E. coli *O157:H7 EDL 933. Bars indicate standard deviation of the mean.*

tested. Shama compared water (UV absorptivity of 0.18) and humic acid solutions with different concentrations (UV absorptivitiy from 0.36 to 4). UV light penetration was markedly affected by the absorption of the UV radiation. The presence of small numbers of particles in a liquid can greatly reduce UV penetration; thus, samples with higher absorptivity values require higher UV doses to inactivate *E. coli.*

Effect of thickness of film exposed to UV radiation. Another factor influencing UV effectiveness as a bactericide is the thickness of the food layer exposed to radiation. According to Tables 2 and 3, *D*-values were higher for thicker films.

When electromagnetic radiation of power P_0 penetrates a liquid, photons and absorbent particles interact, so the transmitted radiation is reduced from P_0 to P. The transmittance T of a solution is the fraction of incident radiation that is transmitted by the solution, i.e., $T = P/P_0$. The Lambert-Beer law states that

$$P = P_0 \exp(-a{\cdot}b{\cdot}c) \qquad (4)$$

where a is the specific absorptivity (liters per mole per centimeter), b is the path of the cuvette (sample holder) (centimeters), and c is the concentration in solution (moles per liter).

The absorbed energy fraction (AEF) for a sample can be defined as

$$\text{AEF} = (P_0 - P)/P_0 = 1 - \exp(-a{\cdot}b{\cdot}c) \qquad (5)$$

where $P - P_0$ is the absorbed energy, P_0 is the incident energy, c is the food concentration, and b is the thickness of the exposed film. Equation 5 gives the relationship between AEF and film thickness for stagnant conditions.

Table 4 shows the calculated AEF values for the stagnant juice films of different thicknesses (0.7 and 2.8 mm) exposed to UV radiation. For a given juice concentration, the product of a and c was considered constant ($a{\cdot}c = cte = \alpha$). A linear relationship was observed between the obtained *D*-values (joules per square centimeter) and AEF values in the different juices studied. This relationship was not modified by the culture medium used nor by the strain tested. There were no significant differences in behavior between pathogenic and nonpathogenic *E. coli* strains (Figs. 6 and 7).

The equation proposed (equation 5) allows the required values of D to be predicted in liquid foods of various ab-

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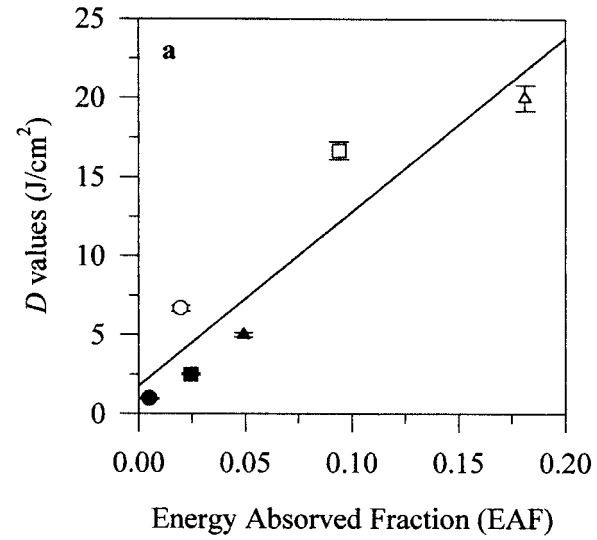
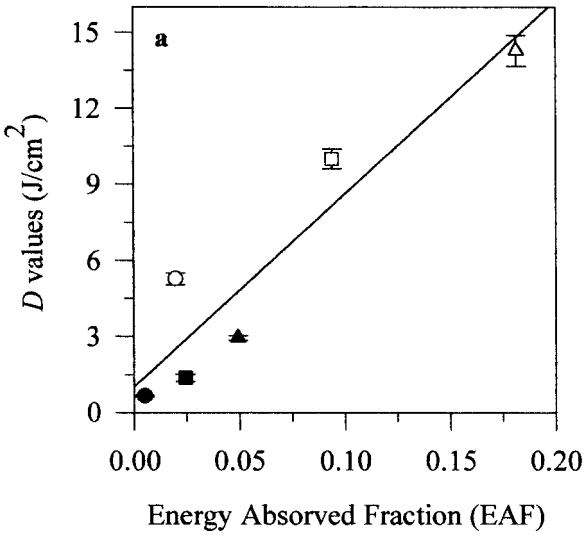
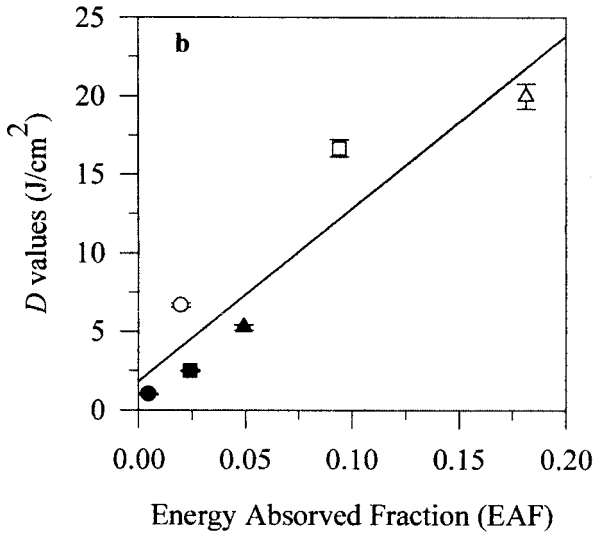
TABLE 4. *Absorbed energy fraction and* D*-values for stagnant juice films of different thicknesses;* D_{TSA} *and* D_{SMAC} *were obtained from TSA and SMAC as recovery media, respectively, for* E. coli *ATCC 25922 and O157:H7 EDL 933*

Absorptivity of fruit juices[a]	Thickness of the stagnant liquid film (mm)	AEF[b]	D_{TSA} (J/cm^2)		D_{SMAC} (J/cm^2)	
			ATCC 25922	O157:H7 EDL 933	ATCC 25922	O157:H7 EDL 933
Orange (α = 0.0715)	0.7	0.005	0.96	0.99	0.65	0.59
	2.8	0.02	6.67	6.67	5.26	5.26
Apple (α = 0.3528)	0.7	0.02	2.50	2.50	1.37	1.31
	2.8	0.09	16.67	16.67	10.00	8.33
Multifruit (α = 0.7230)	0.7	0.05	5.00	5.26	2.94	2.78
	2.8	0.18	20.00	20.00	14.28	14.28

[a] α indicates the absorptivity values.
[b] AEF, absorbed energy fraction.







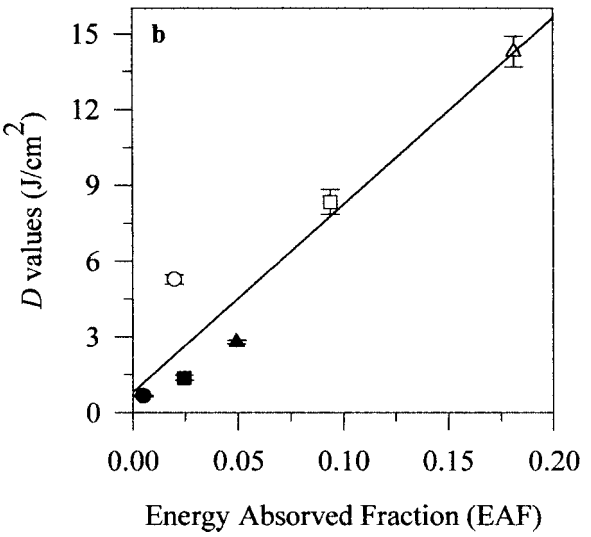


FIGURE 6. *Relationship between* D*-values and the absorbed energy fraction (AEF) in the different juices and different film thicknesses with TSA as recovery medium.* ▲*, multifruit juice;* ■*, orange juice;* ●*, apple juice. Open symbols indicate 2.8-mm-thick films; closed symbols indicate 0.7-mm-thick films. (a)* E. coli *ATCC 25922,* r^2 = 0.87. *(b)* E. coli *O157:H7 EDL 933,* r^2 = 0.88. *Bars indicate standard deviation of the mean.*

FIGURE 7. *Relationship between* D*-values and the absorbed energy fraction (AEF) in the different juices and different film thicknesses with SMAC as recovery medium.* ▲*, multifruit juice;* ■*, orange juice;* ●*, apple juice. Open symbols indicate 2.8-mm-thick films; closed symbols indicate 0.7-mm-thick films. (a)* E. coli *ATCC 25922,* r^2 = 0.88. *(b)* E. coli *O157:H7 EDL 933,* r^2 = 0.90. *Bars indicate standard deviation of the mean.*

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sorptivities (in the studied range) and film thicknesses (0.7 and 2.8 mm) under stagnant conditions. The AEF increases with film thickness, thus *D* was higher for the 2.8-mm film than for the thinner film (0.7 mm).

Sommer et al. *(26)* used different *E. coli* strains inoculated into water and exposed a stagnant film of 4 mm thickness to UV radiation. They obtained a 6-log reduction in bacterial population with UV doses of 0.0012 to 0.012 J/cm^2. For UV radiation to be effective in liquids with high absorptivity, the liquid must be exposed in thin films *(6)* where UV radiation is absorbed less by the liquid and bacteria are more likely to be exposed to lethal doses.

Effect of stirring rate. The resistance of *E. coli* strains in liquid films to UV radiation was influenced by the stirring conditions. Stirring of the thinner films (0.7 mm) resulted in the strongest UV radiation bactericidal effect in all juices. The weakest effect was observed when using stagnant 2.8-mm-thick films for the three juices and both culture media. In each juice studied, stirring of the exposed film reduced *D*-values; stirring of 0.7-mm-thick films resulted in *D*-values between 22.7 and 33 times lower than those for stagnant films of the same thickness. Stirring of 2.8-mm-thick films decreased *D*-values by 2.6 to 4.5 times compared with the values obtained for the same juice under stagnant conditions. This comparison was made with TSA as the recovery medium for the two strains tested.

The use of 0.7-mm-thick films under stirring conditions reduced *D*-values 33.6 to 52 times, depending on the juice, for both strains compared with the same juice in 2.8-mm-thick stirred films. In 0.7-mm-thick stagnant films, *D*-values decreased by 3.8 and 6.9 times, according to the juice used, for both tested strains compared with 2.8-mm-thick films of the same juice under stagnant conditions. Therefore, thickness of the exposed film influenced UV reactions to the extent that microbial growth in 2.8-mm-thick films was practically unaffected. Thus, the higher the thickness of the liquid film exposed to the treatment, the lower the effectiveness of UV radiation.

No significant differences ($P < 0.05$) in *D*-values were found between the two *E. coli* strains tested. Duffy et al. *(8),* working with raw apple cider, observed that the sensitivity of *E. coli* ATCC 25922 to UV light was similar to that of *E. coli* O157:H7 when using TSA as the culture medium.

Several conclusions can be reached based on the data generated in this study. Among the three mathematical models tested to describe behavior of pathogenic and nonpathogenic strains of *E. coli* exposed to UV radiation, the first order kinetics best fit the experimental data, with satisfactory coefficients of determination and lower standard deviations for its fitting parameters compared with those of the other two models: the single hit–single target model and the multitarget model. This result allowed the determination of *D*-values for the different treatments.

No significant differences were observed in the counts of surviving bacteria when they were expressed as dimensionless concentrations (log N/N_0) depending on the inoculum concentration employed.

The most suitable culture medium for the recovery of *E. coli* strains in juices exposed to UV radiation is TSA because it produces higher bacterial counts. The *D*-values (J/cm^2) obtained in all tested juices were higher for TSA than for SMAC as recovery medium for both *E. coli* strains. No significant differences ($P < 0.05$) in *D*-values were found between the two *E. coli* strains tested.

A linear relationship was found between *D*-values and the absorptivity coefficients for all the juices, recovery media, and strains tested. The higher the absorbance of the medium, the greater the dose of UV radiation required to inactivate *E. coli* strains.

An equation was developed to describe the relationships between the fraction of energy absorbed by the system, the thickness of the film exposed to UV radiation, and the absorptivity coefficient of the juices. A linear relationship was found between the *D*-values and the AEF in the different juices tested.

In the three juices analyzed, stirring of the medium exposed to UV radiation and a thinner film (0.7 mm) produced the highest bactericidal effect. Film thickness influenced UV reaction; thus, the use of stagnant 4-mm-thick films resulted in very poor microbial inhibition.

The implications of the present research are limited because only two strains of *E. coli* (O157:H7 EDL 933 and ATCC 25922) were used. However, future research will be be carried out with strain mixtures to test the range of acid resistance and possible UV resistance.

UV radiation was an efficient treatment for juices contaminated with *E. coli* O157:H7. Additional work is now being conducted in our laboratory to test the effect of UV radiation on the quality properties of the juices, such as ascorbic acid concentration, color, and flavor.

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REFERENCES

1. Ahmed, N. M., and D. E. Conner. 1995. Evaluation of various media for recovery of thermally-injured *Escherichia coli* O157:H7. *J. Food Prot.* 58:357–360.
2. Association of Official Analytical Chemists. 1984. Official methods of analysis, 14th ed. Association of Official Analytical Chemists, Washington, D.C.
3. Bell, C., and A. Kyriakides. 1998. *E. coli*—a practical approach to the organism and its control in foods. Blackie Academic Press, New York.
4. Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barret, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA* 269:2217–2220.
5. Clavero, M. R. S., and L. R. Beuchat. 1995. Suitability of selective plating media for recovering heat- or freeze-stressed *Escherichia coli* O157:H7 from tryptic soy broth and ground beef. *Appl. Environ. Microbiol.* 61:3268–3273.
6. Downing, D. L. (ed.). 1989. Processed apple products. Van Nostrand Reinhold, New York.
7. Doyle, M. P. 1991. *Escherichia coli* O157:H7 and its significance in foods. *Int. J. Food Microbiol.* 12:289–302.
8. Duffy, S., J. Churey, R. W. Worobo, and D. W. Schaffner. 2000.

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Analysis and modeling of the variability associated with UV inactivation of *Escherichia coli* in apple cider. *J. Food Prot.* 63:1587–1590.
9. Food and Drug Administration. 2001. FDA publishes final rule to increase safety of fruit and vegetable juices. Available from: http://www.cfsan.fda.gov/~lrd/hhsjuic4.html. Accessed 5 April 2004.
10. Foley, D. M., K. Pickett, J. Varon, J. H. Lee, D. Min, F. Caporaso, and A. Prakash. 2002. Pasteurization of fresh orange juice using gamma irradiation: microbiological, flavor, and sensory analyses. *J. Food Sci.* 67:1495–1501.
11. Gardner, D. W. N., and G. Shama. 1998. The kinetics of *Bacillus subtilis* spore inactivation on filter paperby u.v. light and u.v. light in combination with hydrogen peroxide. *J. Appl. Microbiol.* 84:633–641.
12. Gritz, D. C., T. Y. Lee, P. C. McDonnell, K. Shih, and N. Baron. 1990. Ultraviolet radiation for the sterilization of contact lenses. *CLAO J.* 16:294–298.
13. Huang, Y., and R. Toledo. 1982. Effect of high doses of high and low intensity ultraviolet radiation on the surface microbiological counts and storage life of fish. *J. Food Sci.* 47:1661–1669.
14. Iu, J., G. S. Mittal, and M. W. Griffiths. 2001. Reduction in levels of *Escherichia coli* O157:H7 in apple cider by pulse electric fields. *J. Food Prot.* 64:964–969.
15. Janda, J. M., and S. L. Abbott. 1998. The *Enterobacteriaceae.* Lippincott-Raven Publishers, Washington, D.C.
16. Johnson, R. P., R. C. Clarke, J. B. Wilson, S. C. Read, K. Rahn, S. A. Renwick, K. A. Sandhu, D. Alves, M. A. Karmali, H. Lior, S. A. McEwen, J. S. Spika, and C. L. Gyles. 1996. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli*. *J. Food Prot.* 59:1112–1122.
17. Kuo, F. L., J. B. Carey, and S. C. Ricke. 1997. UV inactivation of shell eggs: effect on population of aerobes, molds, and inoculated *Salmonella typhimurium. J. Food Prot.* 60:639–643.
18. Matttick, L. R., and J. C. Moyer. 1983. Composition of apple juice. *J. Assoc. Off. Anal. Chem.* 66:1251–1255.
19. Morgan, R. 1989. Green light disinfections. *Dairy Ind. Int.* 54:33–35.
20. Parish, M. E. 1997. Public health and nonpasteurized fruit juices. *Crit. Rev. Microbiol.* 23:109–119.
21. Peleg, M., and M. B. Cole. 1998. Reinterpretation of microbial survival curves. *Crit. Rev. Food Sci.* 38:353–380.
22. Riley, L. W. 1987. The epidemiologic, clinical and microbiologic features of hemorrhagic colitis. *Annu. Rev. Microbiol.* 41:383–407.
23. Robinson, J. F. 1971. Making and preserving apple cider. Farmer's Bulletin no. 2125. U.S. Department of Agriculture, Washington, D.C.
24. Sage, J. R., and S. C. Ingham. 1998. Evaluating survival of *Escherichia coli* O157:H7 in frozen and thawed apple cider: potential use of a hydrophobic grid membrane filter-SD 39 agar method. *J. Food Prot.* 61:490–494.
25. Shama, G. 1992. Ultraviolet irradiation apparatus for disinfecting liquids of high absorptivities. *Lett. Appl. Microbiol.* 15:69–72.
26. Sommer, R., M. Lhotsky, T. Haider, and A. Cabaj. 2000. UV inactivation, liquid-holding recovery, and photoreactivation of *Escherichia coli* O157 and other pathogenic *Escherichia coli* strains in water. *J. Food Prot.* 63:1015–1020.
27. Steele, B. T., N. Murphy, G. S. Arbus, and C. P. Rance. 1982. An outbreak of haemolytic uremic syndrome associated with ingestions of fresh apple juice. *J. Pediatr.* 101:963–965.
28. Stermer, R. A., M. Lasater-Smith, C. F. Brasington. 1987. Ultraviolet radiation—an effective bactericide for fresh meat. *J. Food Prot.* 30: 108–111.
29. Weeratna, R. D., and M. P. Doyle. 1991. Detection and production of verotoxin 1 of *Escherichia coli* O157:H7 in food. *Appl. Environ. Microbiol.* 57:2951–2955.
30. Wright, J. R., S. S. Sumner, C. R. Hackney, M. D. Pierson, and B. W. Zoecklein. 2000. Efficacy of ultraviolet light for reducing *Escherichia coli* O157:H7 in unpasteurized apple cider. *J. Food Prot.* 63: 563–567.
31. Zhao, T., M. P. Doyle, and R. E. Besser. 1993. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. *Appl. Environ. Microbiol.* 59:2526–2530.

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