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ORIGINAL PAPER

Ultraviolet Treatment of Orange Juice to Inactivate *E. coli* O157:H7 as Affected by Native Microflora

Juan M. Oteiza · Leda Giannuzzi · Noemí Zaritzky

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Abstract The effect of yeast concentration on ultraviolet (UV) inactivation of five strains of *Escherichia coli* O157:H7 from different sources, inoculated both individually and simultaneously in orange juice, was analyzed and mathematically modeled. The presence of yeast cells in orange juice decreases the performance of UV radiation on *E. coli* inactivation. UV absorption coefficients in the juice increased with increasing yeast concentration, and higher UV doses were necessary to inactivate bacterial strains. UV intensities of I=3.00±0.3 mW/cm^2 and exposure times (t) between 0 and 10 min were applied; radiation doses (energy, $E=I\times t$) ranging between 0 and 2 J/cm^2 were measured using a UV digital radiometer. All the tested individual strains showed higher resistance to the treatment when UV radiation was applied at 4 °C in comparison to 20 °C. UV inactivation of *E. coli* O157:H7 individual strain was satisfactory fitted with a first order kinetic model. A linear relationship was found between UV absorptivities and D values (radiation doses required to decrease microbial population by 90%) for each strain. The dose required to reach 5-log reduction for the most unfavorable conditions that is the most UV resistant strain, and maximum background yeast concentration was 2.19 J/cm^2 at 4 °C (corresponding to 11 min of UV treatment) and 2.09 J/cm^2 at 20 °C (corresponding to 10.55 min of UV treatment). When a cocktail of strains was inoculated in orange juice, the logistic equation was the best model that fits the experimental results due to the deviation from the log-linear kinetics. The UV resistance between strain cocktail and single strain were mathematically compared. Slopes of the decline curves for strain cocktail at high UV doses were lower than the slopes of the log-linear equation calculated for the individual strains, even for the most resistant one. Therefore, microbial inactivation tests using a cocktail of strains are particularly important to determine the performance of the UV inactivation treatment.

Keywords UV radiation · *Escherichia coli* O157:H7 · Orange juice · Yeast concentration · Temperature · Mathematical modeling · D values

J. M. Oteiza (✉) · L. Giannuzzi · N. Zaritzky
Centro de Investigación y Desarrollo en Criotecnología de Alimentos, CIDCA, CCT La Plata—CONICET—Fac. Ciencias Exactas, Universidad Nacional de La Plata,
Calle 47 y 116,
La Plata 1900, Argentina
e-mail: jmoteiza@cidca.org.ar

N. Zaritzky
Fac. de Ingeniería, Universidad Nacional de La Plata,
Calle 47 y 116,
1900 La Plata, Argentina

Introduction

Historically, acid foods such as fruit juices (apple and orange) have been considered safe; however, several foodborne disease outbreaks attributed to unpasteurized juices contaminated with *Salmonella* spp., and *Escherichia coli* O157:H7 have demonstrated that unpasteurized juice can be a vehicle for foodborne diseases (Bull et al. 2004; Sastry et al. 2000). *E. coli* O157:H7 has been implicated as the causative agent in major outbreaks of diarrhea and hemolytic uremic syndrome associated with the consumption of nonpasteurized apple cider and apple juice (CDC 1997; Besser et al. 1993). Parish (1998) and Ryu and Beuchat (1998) reported the presence of *E. coli* in unpasteurized orange juice (pH3.9–4.2), and outbreaks of entherotoxigenic *E. coli* infection associated with orange

juice have been reported by Singh et al. (1995). These findings suggest that nonpasteurized orange juice represents a product with the potential to carry acid-tolerant pathogenic microorganisms such as *E. coli* O157:H7.

Fecal contamination from the use of dropped unwashed fruit has been implicated as the source of *E. coli* in some outbreaks (CDC 1996). However, vectors such as birds could potentially deposit this pathogen on fruits (Wallace et al. 1997).

According to the United States Food and Drug Administration regulation for juice production (USFDA 2001), the application of nonthermal treatment has to assure a 5-$\log_{10}$ reduction in the pertinent pathogen (Murakami et al. 2006). The reduction of *E. coli* O157:H7 is recommended for apple and orange juice with no specific product/pathogen association.

Thermal processing has been widely used for juice pasteurization; however, it may cause substantial changes in flavor and nutritional content of the juices and may be cost prohibitive for many small processing operations. In response to these limitations, other methods of achieving appropriate reductions in pathogenic populations are under examination as possible alternative treatments (Koutchma et al. 2004; Norton and Sun 2008; Walkling-Ribeiro et al. 2008).

Ultraviolet (UV) treatment is a disinfection method that can be applied to inactivate harmful microbes in food (Koutchma 2009; Geveke 2008; Schenk et al. 2008; Tran and Farid 2004). UV inactivation of microorganisms is based on the exposure to UV radiation, with greater effect at wavelengths between 250 and 260 nm (Oteiza et al. 2005). The wavelength of 253.7 nm is most efficient in terms of germicidal effect since photons are absorbed most by the DNA of microorganisms at this specific wavelength (Koutchma 2009).

Treatment with ultraviolet energy offers several advantages to food processors as it does not leave a residue and contributes to energy saving. However, UV radiation has some risks for the eyes and other parts of direct exposure; therefore, the use of eye protection is necessary.

In food processing, UV disinfection of water has been used in different processes such as brewing (McCarty and Scanion 1993), soft drinks (Gibss 2000), cheese-making (Honer 1988). UV radiation has been also used in sterilizing sugar syrup (Stother 1999). In the last years, the application of UV radiation has been focused in the treatment of raw apple cider or fruit juices. Different works have been conducted to analyze the effect of UV on *E. coli* (Koutchma et al. 2007 in orange juice; Guerrero-Beltrán and Barbosa-Cánovas 2006 in apple juice; Oteiza et al. 2005 in orange juice; Basaran et al. 2004 in apple cider; Koutchma et al. 2004 in apple juice; Wright et al. 2000 in apple cider; and Worobo 1999 in apple cider).

The presence of natural microbial flora (acidolactic bacteria, thermo-acidophilic bacteria, molds, and yeasts) can affect the effectiveness of the UV treatment.

The presence of different elements that also absorb UV radiation (particles of pulp in the juice, other microorganisms such as yeasts) increases the necessary doses to inactivate a target microorganism.

Yeast cells are larger in size than bacteria. The presence of yeast increases the absorption coefficient. These reduce the efficacy of the UV treatment.

The effect of UV radiation on bacteria may vary from the species and, in the same species, may depend on the strain (Guerrero-Beltrán and Barbosa-Cánovas 2005). Bachman (1975) reported wide variation in the UV susceptibility levels of different bacteria. Research by Sommer et al. (2000) indicated significant differences in UV resistance between three strains of *E. coli* O157:H7 that were inactivated by UV light in a water system. Data from this study demonstrated that the use of a single strain of a particular species is not adequate for the determination of a specific dose for a given log reduction. EPA Scientific Advisory Panel specifically recommended the testing of five outbreak -related strains in a cocktail for each pathogen (Yaun et al. 2003), and they proposed that the biological variability associated with the observed responses has to be evaluated (Yaun et al. 2003; Wright et al. 2000).

The objectives of this work were (a) to analyze in natural orange juice the effect of UV radiation on five strains of *E. coli* O157:H7 of different origin inoculated both individually and combined in a strain cocktail; (b) to study the effect of the background yeast concentration on the juice absorptivity, considering that yeasts are the main constituents of the natural microflora in orange juice and their large size can affect UV treatment performance; (c) to determine the influence of the temperature at which UV radiation is applied (4 or 20 °C); and (d) to model mathematically the UV inactivation curves of the individual strains and of the five strain cocktail.

Materials and Methods

Orange Juice Preparation

Fresh orange (Valencia var.) were purchased in the local market and stored at 10 °C for 24 h before performing the tests. To obtain fresh orange juice, instruments, equipment, and oranges were washed and brushed manually with sodium hypochlorite solution (0.25 g/kg) and finally with tap water as described by Andrés et al. (2001).

The pH of the juices (obtained by manual squeezing the oranges in the laboratory) was determined by an electrode (model 50215, Hach, Loveland, USA) on a pH

meter (model EC30, Hach, Loveland, USA). The refractive index and soluble solids (°Brix) was determined by an Abbe-type refractometer (Bellingham, BS, England). The effect of UV treatment on these juice properties was measured. All the determinations were done in triplicates.

Aliquots of the juice were stored at 20 °C in order to obtain different ranges of background yeast concentrations (log CFU/ml), Y1=1.00–2.69, Y2=2.70–3.69, Y3=3.70–4.89, and Y4=4.90–6.30, which were enumerated using surface plating in yeast extract, glucose, and chloranphenicol agar (YGC, Merck KGaA, Darmstadt, Germany) and incubated at 25 °C for 5 days. The incubation time to obtain the different yeast concentrations ranged between 30 min and 2.5 days. In all cases, pH values of the juices were not affected by the different yeast concentrations.

Determination of UV Absorptivities of the Juices

Yeast concentration may increase the UV absorption coefficients of the juice. In order to obtain these coefficients, several dilutions of orange juice in sterile water with different yeast concentrations were prepared, and their absorbances at 254 nm were determined in 1-cm light path quartz cuvettes on a spectrophotometer (Beckman, DU 650, USA). The slope of the regression line obtained by plotting absorbance vs. sample concentration (*V*/*V*) was considered as the absorption coefficient; however, this coefficient does not correspond to the molar absorptivity because concentration is not expressed as mol/l.

Inoculation of the Juice with *E. coli* O157:H7 Individual Strains

Five *E. coli* O157:H7 strains from different origins were used in this study: 33/98 (animal isolate), 303/00 (fermented sausage isolation), 547/03 (human isolate), 749/03 (hamburger), and EDL 933 (hamburgers isolate). All the strains were obtained from the National Reference Laboratory (Buenos Aires, Argentina).

Stock cultures were maintained at −80 °C in tryptone soy broth (TSB, Difco, Laboratories, Detroit, MI, USA). Considering that microbial cultures were submitted to freezing conditions (−80 °C), cultures were grown in TSB at 37 °C for 24 h and then transferred to TSB for another 24-h period at 37 °C to reach 9.0 to 9.5 log CFU/ml prior to their use. The microorganisms were transferred two times in order to get an effective recovery of the cold injury *E. coli* cells. All the cultures used for UV inactivation were in stationary phase.

Samples of 99 ml natural orange juice with different yeast concentrations (Y1, Y2, Y3, and Y4) were placed individually in sterile bottles and inoculated with 1 ml of each strain of *E. coli* O157:H7 in order to achieve a concentration ranging between 6.00 and 7.00 log CFU/ml. Inoculated juices were tested to verify the *E. coli* population before UV treatment. Serial dilutions (1:10) were performed with sterile 0.1% peptone water, followed by spread plating of selected dilutions in duplicates on TSA plates, according to Oteiza et al. (2005). Typical colonies were counted after incubation at 37 °C for 24 h expressing results as log CFU/ml.

Inoculation of the Juice with *E. coli* O157:H7 Cocktail of Strains

Aliquots of each individual strain (100 μl) were vortexed and then aseptically combined in a 10 ml of TSB to create a five strain cocktail. Serial dilutions (1:10) were performed with sterile 0.1% peptone water, followed by spread plating of selected dilutions in duplicates on TSA plates. Typical colonies were counted after incubation at 37 °C for 24 h, and log CFU/ml values were calculated to determine the inoculum concentration.

Samples of 99 ml natural orange juice containing two levels of yeast concentrations (minimum, Y1=1.00 to 2.70 log CFU/ml and maximum, Y4=4.00 to 6.30 log CFU/ml) were placed individually in sterile bottles and inoculated with 1 ml of the five *E. coli* O157:H7 strain cocktail in order to achieve two different inoculum concentrations (low (Lc=4.00 log CFU/ml) and high (Hc=6.00 log CFU/ml)).

Effect of Temperature (4 and 20 °C) on UV Treatment

For each assay, 5 ml of each inoculated juice (with individual strains or with the strain cocktail) was placed in sterile Petri dishes (9 cm diameter) separately; thus, a layer of 0.7-mm-thick was obtained; the distance between the sample and the UV lamp was fixed at 15 cm.

A special UV chamber was designed with four UV germicidal lamps (254 nm, UV, Lux 30 W/G30 T8, Philips), mercury low pressure, located on the top of the chamber. To analyze the effect of the stirring velocity, an Orbital Shaker with selectable speed was used. This velocity was set at 220 rpm. A ventilation system was included in the chamber to avoid warming of the samples.

UV assays were performed at two temperatures. For this purpose, the UV equipment was installed in temperature controlled chambers set at 20 or 4 °C.

UV intensity flux or irradiance at 254 nm (*I*), expressed in mW/cm^2, exposures times (*t*, ranging between 0 and 10 min), and the radiation dose (energy, $E=I\times t$, ranging between 0 and 2 J/cm^2) were measured using a UV digital radiometer (Vilber Lourmat, Model VLX-3 W CE, France). The radiometer measures UV light intensity for a specific wavelength (254 nm) using a silicon photo-electric sensor

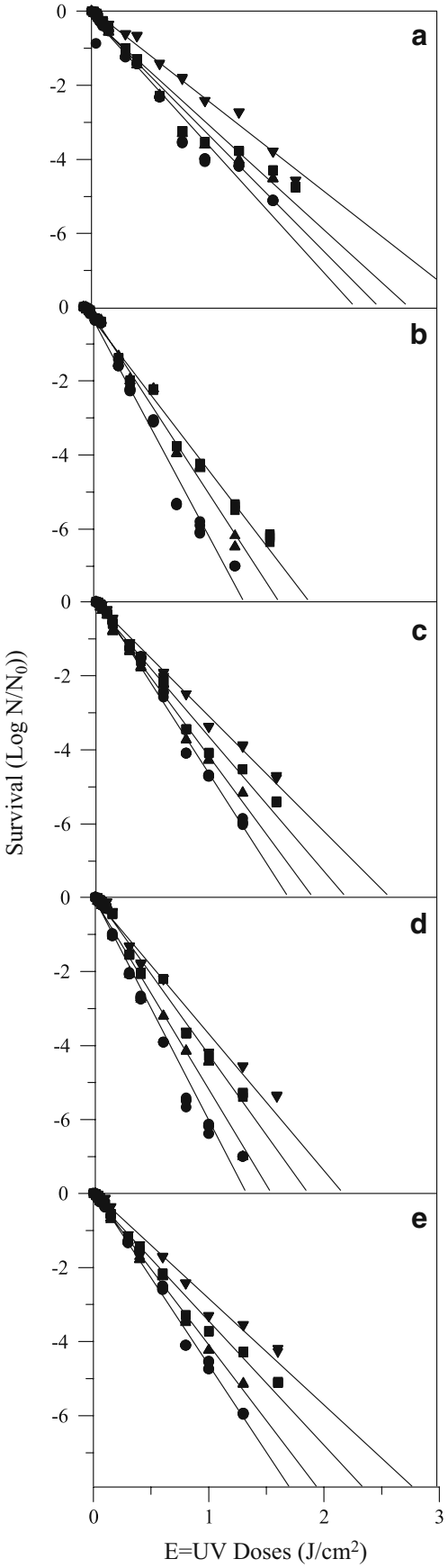

Fig. 1 Effect of UV treatment (254 nm) applied at 20°C on the inactivation of five individual strains of *E. coli* O157:H7 (inoculum concentration: Hc=6.00–7.00 log CFU/ml) in natural orange juice containing different ranges of yeast concentrations: Y1=1.00–2.69 log CFU/ml (*circles*), Y2=2.00–3.69 log CFU/ml (*squares*), Y3=3.69–4.89 log CFU/ml (*triangles*), and Y4=4.90–6.30 log CFU/ml (*inverted triangles*). Strains of *E. coli* O157:H7: **a** 303, **b** 749, **c** 547, **d** 33, and **e** 933. UV radiation doses (*E*) are calculated as the product of the applied intensity (*I*) and the exposure time (*t*)

for a direct measurement of the UV intensity; it is controlled by a microprocessor to measure the UV radiation dosage according to two parameters: time (min or s) or energy (J/cm^2). An average value of I=3.00±0.3 mW/cm^2 was applied in each test.

Enumeration of Surviving *E. coli* O157:H7

Surviving population of *E. coli* 0157:H7 exposed to UV radiation was enumerated by plating on TSA. Appropriate serial dilutions (1:10) were made in 9 ml sterile 0.1% peptone water. In addition, to confirm the presence of *E. coli*, ten colonies were taken for biochemical assays (Mac Faddin 1979).

The period of time demanded by irradiation and sampling was generally less than 20 min. In all cases, there were no differences between the initial blank and final treatment counts after these 20 min. All plates were incubated for 24 h at 37°C and then enumerated. Colonies were counted, and the results were expressed as log CFU/ml; *E. coli* counts were performed in duplicates.

Experimental Design

In the experiments with individual strains of *E. coli* O157:H7, two UV irradiation temperatures (4 and 20°C), four ranges of yeast concentrations (Y1, Y2, Y3, and Y4), and five *E. coli* O157:H7 individual strains (strain 33/98, 303/00, 547/03, 749/03, and EDL 933) were tested using a factorial design. In this case, a total of 40 experimental conditions were analyzed for each UV dose (2 irradiation temperatures×4 levels of yeast concentrations×5 individual strains). In the experiments with strain cocktail of *E. coli* O157:H7, the following factorial design was applied: two UV irradiation temperatures (4 and 20°C), two ranges of yeast concentration (Y1 and Y4), two initial inoculum concentrations of the five *E. coli* O157:H7 strain cocktail (low=(Lc) 4.00 log CFU/ml and high (Hc) 6.00 CFU/ml). Therefore, a total of eight experimental conditions were analyzed for each UV dose (2 irradiation temperatures×2 yeast concentrations×2 inoculum concentrations of the *E. coli* strain cocktail). All the assays were performed in duplicates. In the experiments using individual strains of *E. coli* O157:H7 and in strain cocktail, ten different UV dose ranging between 0 and 2 J/cm^2 were tested.

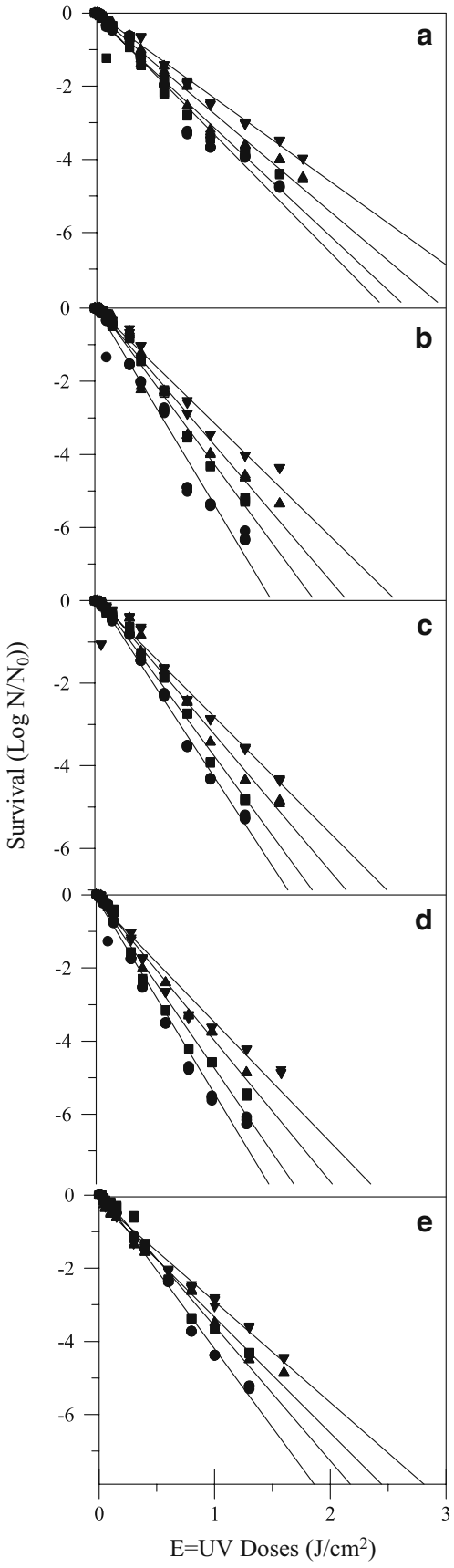

Fig. 2 Effect of UV treatment (254 nm) applied at 4°C on the inactivation of five individual strains of *E. coli* O157:H7 (inoculum concentration: Hc=6.00–7.00 log CFU/ml) in natural orange juice containing different ranges of yeast concentrations: Y1=1.00–2.69 log CFU/ml (*circles*), Y2=2.00–3.69 log CFU/ml (*squares*), Y3=3.69–4.89 log CFU/ml (*triangles*), and Y4=4.90–6.30 log CFU/ml (*inverted triangles*). Strains of *E. coli* O157:H7: **a** 303, **b** 749, **c** 547, **d** 33, and **e** 933. UV radiation doses (*E*) are calculated as the product of the applied intensity (*I*) and the exposure time (*t*)

Mathematical Modeling of the Microbial Inactivation

The following mathematical models were applied to analyze the effect of UV inactivation.

Log-linear model The microbial inactivation of each individual strain caused by UV irradiation is the fundamental principle behind the target theory. This theory is a mathematical approach for the interpretation of survival curves in an irradiation process (Quintero-Ramos et al. 2004) and is given by the following log-linear equation:

$$\log\left(\frac{N}{\mathrm{No}}\right) = -kE = -\frac{E}{D} \tag{1}$$

where N is the microbial counts after applying the UV radiation (J/cm^2) at the radiation dose (E is the radiation dose in J/cm^2), No is the initial microbial counts before UV treatment, N/No is the microbial surviving fraction, and k is the inactivation coefficient.

D values were defined as the radiation doses (J/cm^2) required to decrease microbial population by 90% ($D=1/k$); D values were calculated for the tested strains.

The sigmoidal, three-parameter logistic equation (SigmaPlot 2004 for Windows version 9.0, Systat Inc. 9) that describes a sigmoidal curve with an initial and tailing phase:

$$\log N = \frac{\log \mathrm{No}}{1 + \left(\frac{E}{E_\mathrm{i}}\right)^b} \tag{2}$$

where log(N), log(No), and E have the same meaning as in the previous model. The parameter b (dimensionless) is the Hill Slope that describes the steepness of the curve; it is also known as slope factor or Hill coefficient: If it is positive, the curve increases as E increases, and if it is negative, the curve decreases as E increases. E_i is the dose required to decrease microbial population by 50% (log$N$/logNo=0.5). Besides, the first derivative of the logistic equation (Eq. 2) with respect to the UV doses values (E) (d(log$N$)/dE) was calculated as follows:

$$\frac{\mathrm{d}\log(N)}{\mathrm{d}E} = \frac{-\log(\mathrm{No})(E_\mathrm{i})^{-b} b (E)^{b-1}}{\left(1 + (E/E_\mathrm{i})^b\right)^2} \tag{3}$$

The Gompertz equation and its modified forms have been used primarily in modeling asymmetrical sigmoid shape of microbial growth curves (Linton et al. 1996). Nonlinear survival curves could be modeled as a function of E, using a modified Gompertz equation as follows:

$$\log\left(\frac{N}{\mathrm{No}}\right) = C\exp(-\exp((A+BE)) - C\exp(-\exp(A)) \quad (4)$$

where log (N/No) represents the logarithmic surviving fraction, and the three parameter estimates (A,B,C) represent the different regions of the survival curve: the initial shoulder (A, dimensionless), the maximum slope of the survival curve (B, (cm^2/J)), and the overall change in the survivor number (C, dimensionless).

An alternative description of the survival curves was detailed by Peleg and Cole (1998) using a Weibull-type distribution. They considered that each cell in the population has different resistance to the treatment. There is a spectrum of treatment resistances in the population, and the shape of the survival curves will be determined by the shape of the distribution having different distribution parameters. Survival data were fitted to the cumulative form of the Weibull distribution frequency of the resistances (Peleg and Cole 1998) given by:

$$\log\left(\frac{N}{\mathrm{No}}\right) = -WE^n \quad (5)$$

where, in the original equation, time was replaced by dose (E). W and n are scale and shape factors, respectively. The n, factor describes the shape of the survival curves so that when $n<1$, the survival curve is concave upward (it forms tails), when $n>1$, the survival curves are concave downward (it forms shoulders), and when $n=1$, the survival curve is a straight line on a log scale.

Experimental data were fitted with the described models by linear and nonlinear regressions using Systat software (Systat, Evanston, IL, USA).

Goodness of Fit

To compare the quality of fit of the different microbial inactivation models, the mean square error (MSE; Eq. 6), and the coefficient of determination (R^2) was used.

The smaller the MSE values and the higher the R^2 values, the better the fit of the model to the data (Neter et al. 1996).

$$\mathrm{MSE} = \frac{\sum(\mathrm{PMC}-\mathrm{EMC})^2}{(n-p)} \quad (6)$$

where PMC is the predicted microbial counts(log CFU/ml), EMC is the experimental microbial counts (log CFU/ml), n stands for the number of observations, and p is the number of parameters to be estimated.

Results and Discussion

Effect of Yeast Concentration and UV Treatment Temperature on UV Inactivation of *E. coli* O157:H7 Strains in Orange Juice

The characterization of the orange juice yielded the following results: pH=3.64±0.2, refraction index=1.38, and soluble solids=13.1°Brix. The UV treatments did not modify these values. Absorption coefficients of orange juices containing different yeast levels (Y1 to Y4) ranged between 0.6371±0.0155 and 0.8206±0.0182. A linear increase of these coefficients was caused by the increase of yeast concentrations in orange juice. Differences observed were significant for all yeast concentration ranges ($p<0.05$).

Figures 1 and 2a, b, c, d, and e show surviving microorganism counts of each of the five individual *E. coli* O157:H7 strains inoculated in natural orange juice containing different yeast concentrations (Y1 to Y4) after application of UV treatment both at 20 and 4 °C. Counts of viable microorganisms were plotted as a function of the UV dose (J/cm^2).

Experimental data of the individual strains were satisfactorily fitted with the log-linear model (Eq. 1). Coefficients of determination (r^2) varied between 0.98 and 0.99, and MSE calculated by means of Eq. (6) ranged between 0.014 and 0.058.

Table 1 shows D values for each of the five individual strains of *E. coli* O157:H7 inoculated in orange juice containing different yeast concentrations after UV treatments at 20 and 4 °C.

For all the tested strains, D values increased when background yeast concentration in the juice was higher, indicating a higher UV resistance. This result can be attributed to the higher UV absorptivity values of the juices as yeast concentration increased. Figure 3 shows the linear relationship between the absorptivity values of the juices containing the different tested yeast concentrations and the D values of the five *E. coli* strains at both UV treatment temperatures. Coefficients of determination (r^2) of both regression lines were of 0.95 at 4 °C and of 0.99 at 20 °C.

These results agree with findings of Wright et al. (2000) who indicated that when yeast concentration in apple cider increases, the reduction of the inoculated and UV-treated *E. coli* O157:H7 counts was lower. Kissinger and Willits (1966) reported that UV-induced decrease of microorgan-

Table 1 Effect of the background yeast concentration (Y1 to Y4) and irradiation temperature (4°C and 20°C) on the log-linear inactivation parameter (*D*) of *E. coli* O157:H7 strains inoculated in orange juice treated with UV (254 nm)

E. coli O157:H7 strains	**Yeast concentration in orange juice (log CFU/ml) and irradiation temperature (°C)**															
	Y1=1.00-2.69				Y2=2.70-3.69				Y3=3.70-4.89				Y4=4.90-6.30			
	20°C		4°C		20°C		4°C		20°C		4°C		20°C		4°C	
	D **(J/cm²)**	r^2	***D*** **(J/cm²)**	r^2	***D*** **(J/cm²)**	r^2	***D*** **(J/cm²)**	r^2	***D*** **(J/cm²)**	r^2	***D*** **(J/cm²)**	r^2	***D*** **(J/cm²)**	r^2	***D*** **(J/cm²)**	r^2
303/00	0.296 (0.008)	0.9657	0.311 (0.0008)	0.969	0.3232 (0.0001)	0.964	0.339 (0.0008)	0.972	0.358 (0.001)	0.956	0.375 (0.0008)	0.978	0.418 (0.0006)	0.987	0.439 (0.0005)	0.993
749/03	0.169 (0.003)	0.9841	0.190 (0.0044)	0.977	0.2064 (0.0004)	0.986	0.215 (0.0003)	0.987	0.244 (0.0003)	0.988	0.271 (0.0007)	0.971	0.278 (0.0006)	0.979	0.324 (0.0008)	0.971
547/03	0.210 (0.0003)	0.9923	0.233 (0.0003)	0.992	0.2421 (0.0004)	0.989	0.262 (0.0004)	0.989	0.280 (0.0007)	0.983	0.299 (0.0005)	0.987	0.327 (0.0004)	0.991	0.357 (0.0009)	0.991
33/98	0.168 (0.004)	0.9783	0.191 (0.0050)	0.977	0.1970 (0.0070)	0.974	0.217 (0.0054)	0.9738	0.235 (0.0004)	0.986	0.261 (0.0005)	0.981	0.275 (0.0007)	0.970	0.306 (0.0008)	0.965
EDL 933	0.212 (0.0003)	0.9930	0.233 (0.0003)	0.992	0.2441 (0.0002)	0.995	0.272 (0.0006)	0.9762	0.298 (0.0005)	0.985	0.317 (0.0005)	0.987	0.346 (0.001)	0.962	0.363 (0.0006)	0.986

Values between parentheses are standard deviation of *D*. *D*= radiation doses (J/cm²) required to decrease microbial population by 90%, $k=1/D$

ism counts in maple sap was less effective in the presence of high levels of yeasts.

Guerrero-Beltrán and Barbosa-Cánovas (2005) suggested that the effect of UV radiation on a microorganism may vary from species to species and, in the same species, may depend on the strain. Basaran et al. (2004) reported a wide variation in the UV susceptibility level of different *E. coli* O157:H7 strains in apple cider.

Results shown in Table 1 allow to compare the highest UV resistance of strain 303/00 (isolated from fermented sausage) with that of the lowest resistant strain, 33/98 (animal origin). When the yeast concentration was Y1, the resistance of strain 303/00 was between 67.0% and 84.5% higher than that of strain 33/98 when UV treatment was carried out at 20°C; comparatively, when the treatment was carried out at 4°C, differences were slightly lower (58.7–67.3%).

According to the FDA regulations, the use of alternative treatments to pasteurization should be able to achieve a five log reduction in a pathogen microorganism counts associated to a particular product. From these results, the UV doses required to reach such 5-log (5D) reduction in the case of the most resistant strain of *E. coli* O157:H7 (303/00) was 1.48 J/cm² (corresponding to 7.50 min of UV treatment) at Y1 and a temperature of 20°C. However, when yeast concentration increased to Y4, the same UV dose (1.48 J/cm²) would reduce *E. coli* counts by only 3.54 log cycles at 20°C instead of 5 log cycles. Then, the presence of yeasts can lead to health risks, considering that the infective dose of *E. coli* O157:H7 is very low (100 cells).

The dose required to reach 5-log (5D) reduction for the most unfavorable conditions (*E. coli* 303/00 and yeast concentration Y4) was 2.19 J/cm² at 4°C (corresponding to 11 min of UV treatment) and 2.09 J/cm² at 20°C (corresponding to 10.55 min of UV treatment).

The effect of the temperature at which the UV treatment was carried out (20°C or 4°C) was analyzed by comparing *D* values of the five individual *E. coli* O157:H7 strains (Table 1).

UV treatments carried out at 4°C led to *D* values significantly higher ($p \leq 0.05$) than at 20°C meaning that, all the strains showed higher resistance to UV radiation at the lower temperature. Thus, for the most resistant strain of *E. coli* O157:H7 (303/00) in orange juice, with a yeast concentration Y1, the energy required to reduce the *E. coli* population by 5 log counts was 1.56 J/cm² at 4°C (corresponding to 7.90 minutes of UV treatment), higher than the value of 1.48 J/cm² required at 20°C (corresponding to 7.48 minutes of UV treatment). Thayer and Boyd (2001) working with ionizing radiation reported that low temperatures increased bacterial resistance to radiation due to the decrease in the formation and mobility of free radicals produced in the food, which are responsible of the cell destruction. A similar effect may occur during

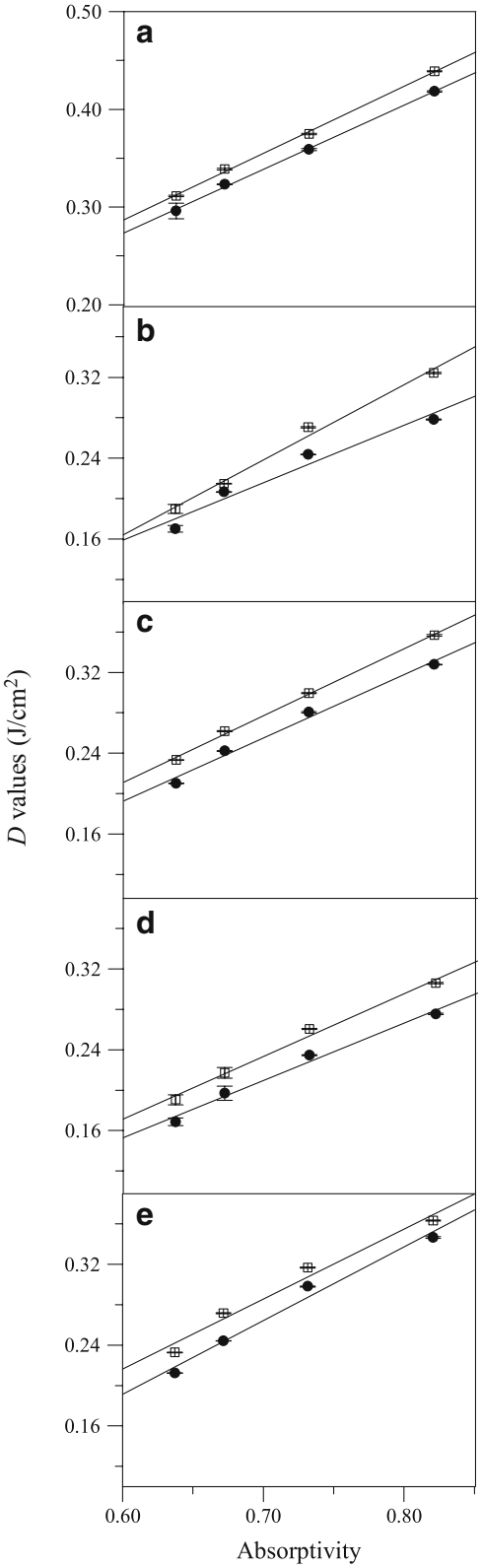

Fig. 3 Linear relationship between absorptivity coefficients of natural orange juice containing different yeast concentrations and *D* values of the following *E. coli* O157:H7 strains: **a** 303/00, **b** 749/03, **c** 547/03, **d** 33/98, and **e** EDL 933 at two different UV treatment temperatures: 4°C (*squares*) and 20°C (*circles*). *Bars* show the average standard deviation

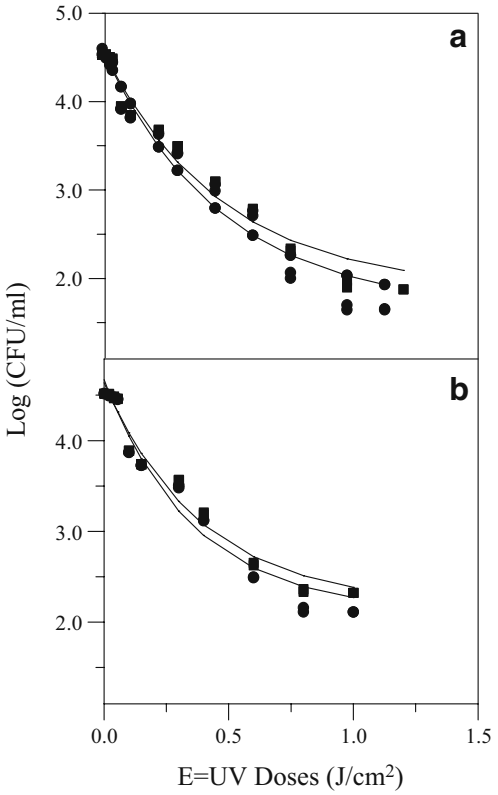

Fig. 4 Effects of yeast concentration and UV doses applied at both treatment temperatures (4 or 20°C) on inactivation of five *E. coli* O157:H7 strains inoculated simultaneously (cocktail) with low inoculum concentrations (Lc=4.00 log CFU/ml) in natural orange juice. **a** Corresponds to a range of high yeast concentrations (Y4= 4.90–6.30 log CFU/ml). **b** Corresponds to a range of low yeast concentrations (Y1=1.00-2.69 log CFU/ml). UV applied treatment temperature: 4°C (*circles*) and 20°C (*squares*). *Full lines* correspond to the logistic model

UV treatment of food. UV light can adversely affect food by generating free radicals in products by a wide variety of organic photochemical reactions (Koutchma 2009). However, in this case, the main agent for cell destruction after UV treatment is mainly due to the formation of pyrimidine dimers in the genome DNA.

Effect of UV Radiation on the *E. coli* 0157:H7 Strains Cocktail in the Orange Juice

Figure 4a, b and Fig. 5a, b, c, and d show the effect of UV radiation at 4°C and 20°C, on the five strain cocktail inoculum of *E. coli* O157:H7. Two ranges of *E. coli* inoculum (low=Lc=4.00 log CFU/ml and high=Hc=6.00 log CFU/ml) and two ranges of yeast concentrations (low= Y1=1.00 to 2.70 log CFU/ml and high=Y4=4.90 to 6.30 log CFU/ml) were tested.

Inactivation of the *E. coli* O157:H7 strains cocktail as a function of UV dose, deviated from the log-linear equation

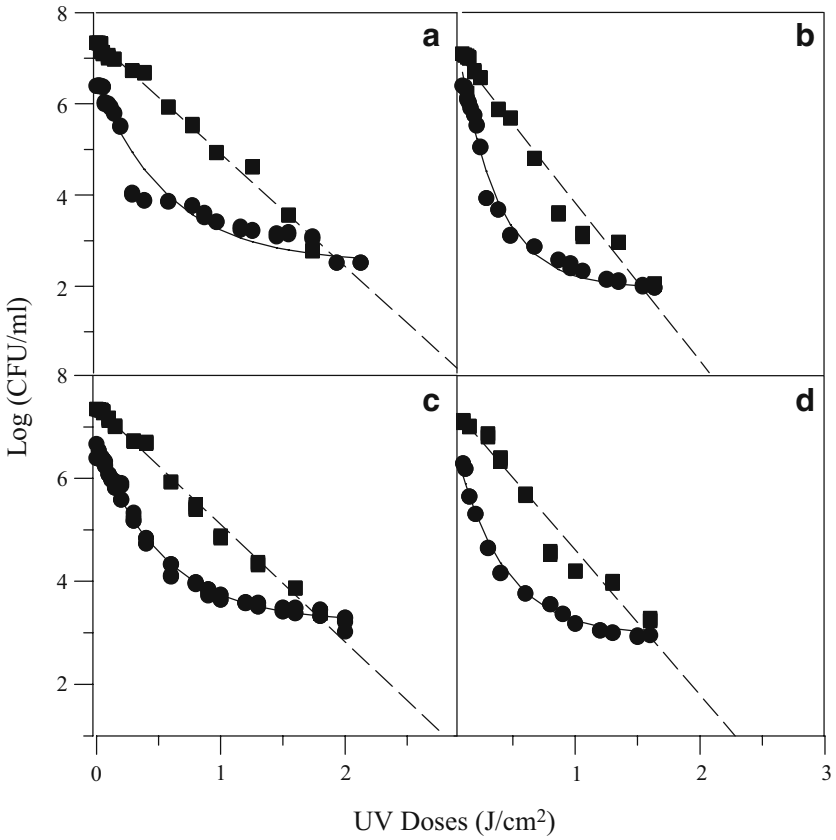

Fig. 5 Effects of yeast concentration and UV doses applied at both treatment temperatures (4 or 20°C) on UV inactivation of five *E. coli* O157:H7 strains inoculated simultaneously (cocktail) with high inoculum concentrations (Hc=6.00 log CFU/ml) in natural orange juice. **a** and **c** Correspond to a range of high yeast concentrations (Y4= 4.90–6.30 log CFU/ml). **b** and **d** Correspond to a range of low yeast concentration (Y1=1.00-2.69 log CFU/ml). **a** and **b** Correspond to UV temperature treatment of 20°C. **c** and **d** Correspond to UV temperature treatment of 4°C. *Circles E. coli* O157:H7 five strain cocktail, *squares* individual more resistant strain (303/00). *Full lines* correspond to the logistic model, and *dotted lines* correspond to the regressions of the individual strains

as shown in Fig. 4. Different nonlinear mathematical equations, logistic, modified Gompertz, and Weilbull, were applied to select the model that shows the best agreement with the experimental results. Among the three nonlinear models, the logistic equation (Eq. 2) consistently produced the best fit to all survival curves (Fig. 4). The MSE values (mean square error, Eq. 6) of the logistic model ranged between 0.017 and 0.050; these values were lower than those obtained by Gompertz equation (between 0.019 and 0.655) and Weilbull model (between 0.018 and 0.08; Table 2).

The slope of the logistic equation (Eq. 3) represents the effect of the UV dose on the microbial population; this slope is not constant for the strain cocktail and depends on log(No), b, and E_i.

Table 3 lists the parameters obtained by applying the logistic model. For the strain cocktail, the slope of the curve

Table 2 Mean square error (MSE) values obtained for different mathematical models (logistic, Gompertz, and Weibull distribution) according to the inactivation of the *E. coli* O157:H7 strains cocktail

E. coli cocktail inoculum level	*T* (°C)	Yeast concentration	MSE values for different mathematical models		
			Logistic	Modified Gompertz	Weibull
Hc	20	Y4	0.020	0.080	0.022
Hc	4	Y4	0.017	0.019	0.018
Hc	20	Y1	0.050	0.066	0.061
Hc	4	Y1	0.018	0.037	0.032
Lc	20	Y4	0.050	0.655	0.051
Lc	4	Y4	0.031	0.052	0.068
Lc	20	Y1	0.030	0.048	0.080
Lc	4	Y1	0.019	0.051	0.053

Hc high inoculum level of the five strains cocktail of *E. coli* O157:H7 (6.00 log CFU/ml), *Lc* low inoculum level of the five strains cocktail of *E. coli* O157:H7 (4.00 log CFU/ml), *Y4* high level of yeast (4.90–6.30 log CFU/ml), *Y1* low level of yeast (1.00–2.69 log CFU/ml)

Table 3 Effect of the temperature at which UV treatment was applied (20 or 4 °C) and influence of yeast concentration (Y1 or Y4) on the logistic equation parameters for two inoculum concentrations of the five *E. coli* O157:H7 strain cocktail (high, Hc and low, Lc), inoculated in orange juice

E. coli cocktail inoculum level	*T* (°C)	Yeast concentration	Parameters of the logistic equation		
			LogNo (log CFU/ml)	*b*	E_i (J/cm^2)
Hc	20	Y4	6.93±0.21	0.69±0.06	0.99±0.11
Hc	4	Y4	6.79±0.13	0.76±0.05	1.45±0.08
Hc	20	Y1	6.86±0.20	0.86±0.05	0.41±0.04
Hc	4	Y1	6.81±0.14	0.95±0.05	0.58±0.04
Lc	20	Y4	4.44±0.07	1.24±0.10	0.97±0.05
Lc	4	Y4	4.53±0.06	1.05±0.08	1.11±0,05
Lc	20	Y1	4.61±0.09	0.99±0.08	0.76±0.04
Lc	4	Y1	4.64±0.09	0.90±0.08	0.90±0.05

Hc high inoculum level of the five strains cocktail of *E. coli* O157:H7 (6.00 log CFU/ml), *Lc* low inoculum level of the five strains cocktail of *E. coli* O157:H7 (4.00 log CFU/ml), *Y4* high level of yeast (4.90–6.30 log CFU/ml), *Y1* low level of yeast (1.00–2.69 log CFU/ml), E_i UV doses that leads to log*N*=logNo/2

calculated with Eq. 3 decreased as the applied dose (*E*) increased (Fig. 4).

For Hc and Lc *E. coli* concentrations, the highest values of the slopes were observed in orange juice with the lowest yeast concentration (Y1). Slope values of the curves represent the effect of UV dose on the survival of *E. coli*. The highest values of the slopes correspond to a higher UV performance (lower microbial resistance to the treatment) and were observed in orange juice containing the lowest yeast concentrations (low absorption coefficient values). This would confirm that yeasts prevent *E. coli* inactivation by increasing the UV absorption coefficients in the juice leading to a weaker inactivation effect on *E. coli.* Such effect was observed regardless of the temperature at which the UV treatment was applied. For the different yeast concentrations, the values of the slopes were higher at 20 °C than at 4 °C; this means that lower temperature treatment increases the resistance to UV radiation.

Figure 5a, b, c, and d allows to compare inactivation curves corresponding to the high level of *E. coli* O157:H7 inoculum in the cocktail, with the straight line of the most UV radiation-resistant individual strain (303/00) submitted to the same UV treatment temperatures and with the same yeast concentrations present in the orange juice. As can be observed, at high UV doses (long exposure times), the cocktail showed a lower slope (tail of the curve) than that of the most resistant individual strain (303/00).

The nonlinear behavior of the five *E. coli* O157:H7 strain cocktail exposed to UV radiation can be analyzed considering that multiple strains may differ in their susceptibility to UV light producing the tailing effect, as demonstrated by Sommer et al. (2000).

Besides, the initial exposure of bacteria to UV is believed to damage the most sensitive cells; as increasing UV doses are received, mutations arise in the DNA code that impede cellular replication; thus, cellular death occurs because neighboring pyrimidine bases begin to form cross-linkages (Sastry et al. 2000). Other causes to the nonlinear behavior are the varying abilities of cells to repair DNA mutation (Miller et al. 1999).

Conclusions

The presence of yeast cells in orange juice decreases the performance of UV radiation on *E. coli* O157:H7 inactivation. UV absorption coefficients in the juice increased with increasing yeast concentration, and higher UV doses were necessary to inactivate *E. coli* O157:H7 strains.

All the tested *E. coli* O157:H7 individual strains showed higher resistance to the treatment when UV radiation was applied at 4 °C in comparison to 20 °C. UV inactivation of *E. coli* O157:H7 individual strain was satisfactory fitted with a first-order kinetic model. A linear relationship was found between UV absorptivities and *D* values for each tested strain, both at 4 and 20 °C. The dose required to reach 5*D*-log reduction for the most unfavorable conditions (the most UV resistant strain, *E. coli* 303/00 and maximum yeast concentration, 4.90–6.30, log CFU/ml) was 2.19 J/cm^2 at 4 °C (corresponding to 11 min of UV treatment) and 2.09 J/cm^2 at 20 °C (corresponding to 10.55 min of UV treatment).

In experiments with the five *E. coli* 0157:H7 strain cocktail, the logistic model yielded the best fit. The highest values of the curve slopes were observed for juices containing the lowest yeast concentrations, confirming again that yeasts are preventing *E. coli* inactivation, by increasing the UV absorption coefficient, independent of the temperature at which the UV treatment was applied.

When a cocktail of strains was inoculated in orange juice, the slopes of the decline curves at high UV doses were lower than the slopes of the log-linear equation calculated for the individual strains, even for the most resistant one (303/00). Therefore, microbial inactivation tests using a cocktail of strains are particularly important to determine the performance of the UV inactivation treatment and the required doses for the reduction of *E. coli* O157:H7 counts in natural orange juice.

The UV treatment may be a practical way in preserving fresh fruit juice longer. Complementary work must be done to show if the main quality parameters (such as ascorbic acid content, color, and enzyme activity) are better preserved by UV treatment than by a thermal process.

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