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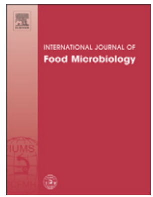

Modeling inactivation kinetics of liquid egg white exposed to UV-C irradiation

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ABSTRACT

The efficiency of UV-C irradiation as a non-thermal pasteurization process for liquid egg white (LEW) was investigated. LEW inoculated with *Escherichia coli* K-12 (ATCC 25253), pathogenic strain of *Escherichia coli* O157:H7 (NCTC12900) and *Listeria innocua* (NRRL B33314) were treated with UV light using a bench top collimated beam apparatus. Inoculated LEW samples were exposed to UV-C irradiation of known UV intensity of 1.314 mW/cm^2 and sample depth of 0.153 cm for 0, 3 5, 7, 10, 13, 17 and 20 min. The populations of *E. coli* K-12, *E. coli* O157:H7 and *L. innocua* were reduced after 20 min of exposure by 0.896, 1.403 and 0.960 log CFU respectively. Additionally, the inactivation data obtained for each strain suspended in LEW was correlated by using Weibull (2 parameter), Log-Linear (1 parameter), Hom (2 parameter) and modified Chick Watson (2 parameter) models. The inactivation kinetics of *E. coli* K-12, *E. coli* O157:H7 and *L. innocua* were best described by modified Chick Watson model with the smallest root mean squared error (RMSE) ($R^2 \geq 0.92$).

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1. Introduction

Liquid egg products (LEP) are very susceptible to spoilage bacteria due to their high nutritive contents. The most important pathogenic microorganisms, which cause several foodborne illnesses in LEP are *Salmonella enteridis*, *Salmonella seftenberg* and *Listeria monocytogenes* (Ferreira and Mastro, 1998; Lee et al., 2001; Ngadi et al., 2003; Mañas et al., 2003). Therefore the use of liquid egg white (LEW) requires an application of a preservation method to inactivate food borne pathogens. Although thermal pasteurization is an effective method of killing microorganisms; it causes some undesirable effects on the volume, quality and functional properties of the LEW (Hamid-Samimi and Swartzel, 2007; Herald and Smith, 1989; Ponce et al., 1999; Gongora-Nieto et al., 2003; Hermawan et al., 2004).

Alternative non-thermal processes including pulsed electric fields (PEF), ultrasound, high hydraulic pressure, oscillating magnetic field, and ionizing radiations have been proposed as a substitute for heat pasteurization of LEW (Bazhal et al., 2006; Jeantet et al., 1999; Lee et al., 2003; Mañas et al., 2003; Ponce et al., 1998; Rasgoti, 2003; Wrigley and Llorca, 1992; Lado and Yousef, 2002). UV-C irradiation, which has the short range germicidal effect on microbial populations at 200–280 nm, is increasingly being proposed as an alternative technology especially for disinfection of transparent liquid foods and decontamination of solid food surfaces. The inactivation mechanism is related to the absorption of UV photons by DNA or RNA pyrimidine bases. The incident light causes a pyrimidine dimer formation on the same DNA strand between two adjacent nucleotides leading to inhibition of transcription and replication and eventually death of the cell (Bolton and Linden, 2003; Koutchma, 2009).

Research is still needed to identify pathogens and their surrogate microorganisms resistant to UV light to ensure microbiological effectiveness and appropriate process design (Sastry et al., 2000). There are few studies cited in the literature about the efficacy of UV-C light for the reduction of pathogenic and spoilage microorganisms in LEW (Ngadi et al., 2003; Geveke, 2008; Unluturk et al., 2008).

The objective of this study is to investigate the efficiency of UV-C irradiation as a non-thermal pasteurization process for LEW with a non-pathogenic strain of *E. coli* K-12 (ATCC 25253), pathogenic strain of *E. coli* O157:H7 (NCTC12900) and *L. innocua* (NRRL B33314) as the target microorganisms using a collimated beam apparatus. For this purpose, inoculated LEW samples were exposed to a UV-C irradiation of a known intensity level of 1.314 mW/cm^2 and a sample depth of 0.153 cm for 0, 3, 5, 7, 10, 13, 17 and 20 min. Weibull, Log-Linear, Hom and modified Chick Watson models have also been applied to describe inactivation kinetics of these microorganisms.

2. Materials and methods

2.1. Sample preparation and properties

Pasteurized LEW purchased from an egg product manufacturer (IPAY Izmir Pastörize San. and Tic. A.Ş., Izmir, Turkey) were selected since pasteurized samples do not have significant levels of indigenous

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Nomenclature

LEP	Liquid egg products
LEW	Liquid egg white
UV	Ultraviolet
N	Microbial population after UV exposure (CFU/ml)
N_0	Initial inoculation level (CFU/ml)
t	UV exposure time (min)
t_R	Reliable time in Weibull model (min)
α	Scale parameter in Weibull model (min)
β	Shape parameter in Weibull model
k	First order rate constant in Log-Linear model ($time^{-1}$)
D	Reciprocal of the first order rate constant (min)
C	Concentration of disinfectant in Chick Watson model
n	Reaction order in Chick Watson model
k_1 and k_2	Characteristic kinetic constants in Chick−Watson model ($time^{-1}$)
k′	Kinetic constant in Hom model ($time^{-1}$)
h	A constant in Hom model
RMSE	Root mean squared error
cal	Calculated data
CFU	Colony forming unit
exp	Experimental data
n	Number of data points for each strain
A_e	Absorbance coefficient at 254 nm wavelength

microflora (<10^2 CFU/ml based on Turkish Food Codex). Background flora of pasteurized LEW samples was additionally checked by surface plating on Tryptic Soy Agar (TSA, Merck, Darmstadt, Germany) for the enumeration of total aerobic bacteria and Violet Red Bile Agar (VRBA, Merck, Darmstadt, Germany) to determine the number of coliforms before UV treatment. The results are presented in Table 1. Physical properties of pasteurized LEW were also measured and summarized in Table 1. Absorbance values of LEW samples were determined by means of a Cary 100 UV–Visible Spectrophotometer (Varian, USA) adjusted to 254 nm using 1 cm quartz cuvette. A variety of dilution factors were applied (1:50, 1:100, 1:250, 1:500 and 1:1000). Absorbance coefficient (A_e) was estimated from the slope of absorbance versus sample concentration plot.

2.2. Biodosimetric study

2.2.1. Target microorganisms (biological dosimeters) and growth conditions

The non-pathogenic strain of *E. coli* K-12 (ATCC 25253), pathogenic and non-toxigenic *E. coli* O157:H7 (ATCC 700728, NCTC 12900) and *L. innocua* (NRRL-B 33314) were used as biological dosimeters. Serotype *E.coli* O157:H7 (ATCC 700728) was obtained from Dr. Ali Aydın, (Department of Food Hygiene and Technology, Faculty of Veterinary, Istanbul University, Turkey) and *L. innocua* (NRRL-B 33314) was provided by Dr. Ayse Handan Baysal (Department of Food Engineering, Izmir Institute of Technology, Turkey). During this study, *E. coli* O157:H7 (ATCC 700728, NCTC 12900) and *L. innocua* (NRRL-B 33314) were cultured from tryptic soy agar (TSA, Merck, Darmstadt, Germany) slanted stock culture, whereas *E. coli* K-12 (ATCC 25253) was cultured from −80 °C lyophilized vials. A broth subculture was enriched after inoculation of a loopful from stock culture into a test tube containing nutrient broth (NB, Merck, Darmstadt, Germany) and strains were incubated overnight (18–24 h) at 37 °C. Incubation for 24 h allowed the respective bacteria to approach stationary phase of growth at a concentration of ca. 8 log unit CFU/ml.

2.2.2. UV irradiation equipment and inactivation treatments

Inoculated LEW samples were exposed to UV-C irradiation using a collimated beam apparatus described in Bolton and Linden (2003) which is shown in Fig. 1. The bench top system consisted of a pair of 15 W low pressure mercury vapor UV lamp (1) at 254 nm wavelength (UVP XX-15, UVP Inc., CA, USA) and a platform (2) on which Petri dish and a vortex mixer (6) (IKA, Yellowline TTS 2, IKA® Werke GmbH & Co. KG, Germany) were placed for UV exposure. A tray system (3) was used in order to raise or lower the Petri dishes, a hole (4) was opened on top of the system with dimensions of one standard Petri dish diameter, a shutter (5) was used to block or allow passage of UV energy.

The system was closed by a front cover during UV experiments to prevent the escape of UV light. The whole system was constructed by cardboard with a flat black paint in order to minimize the loss of light. Samples were placed in Petri dishes directly below the collimated UV beam and stirred continuously during irradiation with a vortex mixer set at 200 rpm. The UV intensity at the surface of the sample (incident intensity (I_o) or irradiance at the surface) was measured using a radiometer with UVX-25 sensor (UVX, UVP Inc., CA, USA). The radiometer was placed at a similar distance as the LEW samples. The UV lamp was switched on for about 30 min prior to UV treatment of LEW samples to reduce fluctuations in UV light. Before usage, the collimated beam apparatus was cleaned and sanitized with 70% (v/v) ethanol.

LEW was added to 50 mm standard Petri dishes to obtain a sample depth of 0.153 cm. The depth was calculated from the ratio of the sample volume and the surface area of a Petri dish. An adequate stirring was applied during treatment in order to ensure equal distribution of UV dose through the sample. The edge effects caused by stirring are avoided by using the smallest possible sample volume (Bolton and Linden, 2003). Biodosimetric study of *E. coli* K-12 (ATCC 25253), pathogenic and non-toxigenic *E. coli* O157:H7 (ATCC 700728, NCTC 12900) and *L. innocua* (NRRL-B 33314) were carried out by direct UV light, exposed on 0.153 cm sample depth at a 1.314 mW/cm^2 UV incident intensity level

Table 1
Microbiological quality and physical properties of LEW.

Property	Value
Total aerobic count (log CFU/ml)	2.10–5.48
Total coliform (log CFU/ml)	2.00–4.30
Density (g/cm^3)	1.030
pH	6.57 ± 0.02
Total solid %	11.50 ± 0.06
Absorbance coefficient (A_e) (cm^{-1})	323.15 ± 13.00
Turbidity (NTU)	776 ± 5.49
Brightness (L*)	23.97 ± 0.23
Redness–greenness (a*)	−1.32 ± 0.07
Yellowness–blueness (b*)	−0.76 ± 0.15

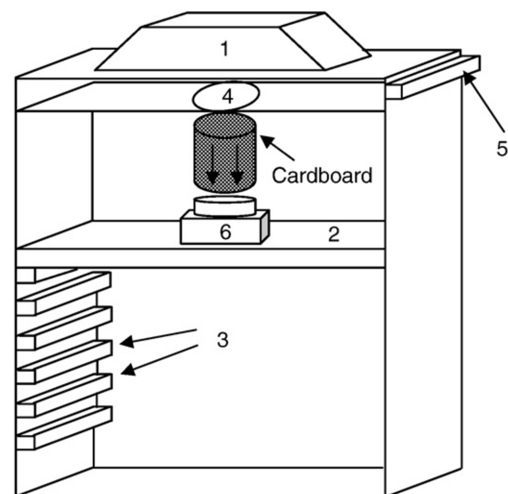

Fig. 1. Laboratory scale closed bench top collimated beam ultraviolet system.

for 3, 5, 7, 10, 13, 17 and 20 min excluding untreated control sample. All studies were conducted within the UV dose range, calculated by the product of incident intensity and exposure time, of 0–26.44 mJ/cm^2. The calculation method of UV dose is described in details by Unluturk et al. (2008). LEW samples were directly inoculated with the subculture to provide a final inoculum of ~10^7–10^8 CFU/ml. For enumeration, appropriate dilutions were made with 0.1% peptone water with phosphate buffered (Merck, Darmstadt, Germany) and surface plated in duplicate on Tryptic Soy Agar (TSA, Merck, Darmstadt, Germany). Sorbitol McConkey agar (SMAC, Merck, Darmstadt, Germany) was used as selective media of *E. coli* O157:H7 (ATCC 700728, NCTC 12900). In order to determine the initial load of microorganisms in LEW samples before UV application, samples were streaked on Violet Red Bile Agar (VRBA, Fluka, Biochemica, Spain) and pour plated using Plate Count Agar (PCA, Fluka, Biochemica, Spain) plates. All the plates were incubated at 37 °C for 24 h and counted. PCA poured plates were incubated at 30 °C for 24–48 h. The enteric population on VRBA plates for tested samples except the one used for *L. innocua* studies was in the limit allowed by Turkish Food Codex (2008).

2.3. Modeling of UV-C inactivation kinetics

2.3.1. Weibull model

A bacterial strain may provide different shapes of inactivation curves due to heterogeneity among the cells of a population even if the population is pure. Weibull distribution is offered as a simple model for description of microbial inactivation by thermal and non-thermal treatment methods. In the case of UV light treatment, inactivation curves are generally sigmoidal and exhibit concavity or convexity behaviors through downwards or upwards as a function of inactivation time or UV dose. The Weibull model usually describes this behavior very well. Weibull distribution function is composed of two parameters (α and β) given in the following correlation (Van Boekel, 2002; Bialka et al., 2008):

$$\log_{10}\left(\frac{N}{N_0}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \tag{1}$$

In the case of $\beta = 1$, the inactivation curve appears first order linearly oriented. Upward concavity of the inactivation curve ($\beta < 1$) indicates stress adaptation of target microorganism, survived after UV exposure. Downward concavity ($\beta > 1$) shows accumulated damaging rates of UV in the cells. The reliable time (t_R) is estimated from these parameters. t_R is the reliable life time indicating 90% reduction of the number of microorganism (Van Boekel, 2002). It is similar to the D value (the time necessary to reduce the number of microorganisms by a factor of 10) but it shows high dependency on β. t_R for UV inactivation is calculated using Eq. (2) (Van Boekel, 2002; Bialka et al., 2008);

$$t_R = \alpha(2.303)^{\frac{1}{\beta}} \tag{2}$$

2.3.2. Log-Linear model

Log-Linear model has been widely accepted and used to describe the microbial inactivation resulted from application of both thermal and non-thermal processes. This model assumes that death of microorganisms follows the rule of first order kinetics. The model is given in the following equation (Van Boekel, 2002):

$$\log_{10}\left(\frac{N}{N_0}\right) = -kt \tag{3}$$

Classical D value is calculated from the reciprocal of the first order rate constant ($D = 2.303/k$, units in min). Eq. (3) is also known as Chick Watson linear equation (Marugán et al., 2008).

2.3.3. Modified Chick Watson model

In the application of disinfection processes such as UV-C irradiation, the inactivation mechanism is very complex and kinetic analysis can be performed using empirical equations. The Chick Watson equation is an empirical disinfection model which considers the rate of inactivation of a target microorganism as an exponential function of the concentration of disinfectant agent (Chick, 1908). General expression of this model is given by;

$$\left(\frac{dN}{dt}\right) = -kC^nN \tag{4}$$

Integrated as a function of time (t), Eq. (4) becomes;

$$\left(\frac{N}{N_0}\right) = \exp\left(-kC^nt\right) \tag{5}$$

In photocatalytic processes with artificial light, the disinfectant concentration could be considered to be constant with time for a fixed configuration of photoreactor lamps. As a result, the equation is reduced to Log-Linear model (Eq. (3)). However, this model is only valid for the description of the linear part in the inactivation curve (Fig. 2). In order to describe the shoulder or tail part in the inactivation curve, the Chick Watson model could be modified by introducing two parameters in Eq. (5) (modified Chick Watson equation) (Marugán et al., 2008):

$$\log\frac{N}{N_0} = -k_1\left[1 - \exp(-k_2t)\right] \tag{6}$$

where k_1 and k_2 are characteristic kinetic constants where k_1 is the inactivation rate constant (min^{-1}), and k_2 is the first order UV decay constant (min^{-1}).

2.3.4. Hom model

Although this model is similar to the Chick Watson model (Eq. (4)), it is a non linear equation incorporating another parameter called h. The expresion is:

$$\log\left(\frac{N}{N_0}\right) = -k^{\prime}t^h \tag{7}$$

When $h = 1$, this equation simplifies to Log-Linear equation (Eq. (3)). For the case $h < 1$ the equation permits the fitting of the tailing (inward concavity) part of the inactivation curve, for $h > 1$, the shoulder (outward convexity) part can be predicted (Marugán et al., 2008). Smaller k′ value (inactivation rate constant) indicates that the microorganism shows larger resistivity to applied treatment.

2.3.5. Model comparison and statistical analysis

Triplicate treatments were carried out and plate counts were performed in duplicate. The results were expressed as average ± standard deviation. The parameters in the Weibull distribution, Log-Linear, Hom equation and modified Chick Watson equation were estimated using only two experimental data sets by a program written in MATLAB® (MATLAB® 6, Mathworks Inc., Natic MA, USA) to find the best fit. The third experimental data set was used to validate the models.

In order to determine the suitability of fitted functions on inactivation data, the root mean squared error (RMSE) was computed using the calculated (cal) and experimentally (ex) determined logarithmic reductions by the following correlation (Eq. (8));

$$RMSE = \sqrt{\frac{1}{n}\sum\left[\left(\log\frac{N}{N_0}\right)_{\text{cal}} - \left(\log\frac{N}{N_0}\right)_{\text{exp}}\right]^2} \tag{8}$$

The smaller the RMSE values, the better the model fits data.

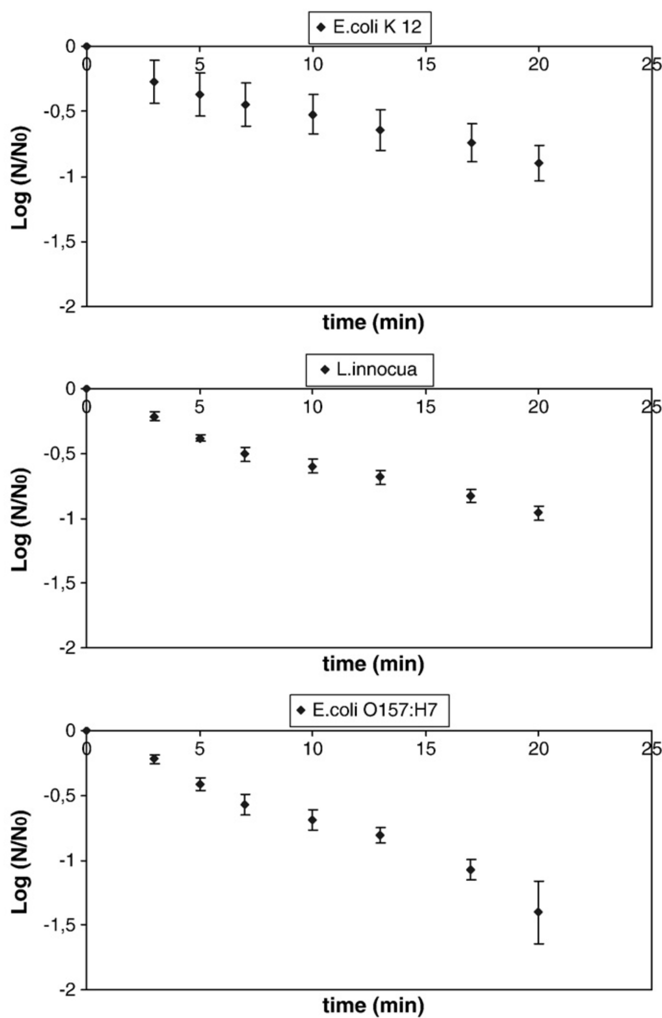

Fig. 2. Inactivation rate results of *E. coli* K-12, *L. innocua* and *E. coli* O157:H7.

Analysis of variance (ANOVA, p = 0.05) was performed by using commercial spread sheet (Microsoft Excel, Redmond, WA, USA) in order to determine the significance of exposure time on α and β (Weibull parameters). Additionally, one way of ANOVA (p = 0.05, time effect) was applied for the log reduction data in order to check whether the values obtained for each microorganisms are significantly different from each other at a prescribed exposure time.

Table 2
Average logarithmic reduction levels of each strain after UV exposure.

t (min)	UV dose (mJ/cm^2)	Reduction (Log) *E.coli* K12	*E.coli* O157:H7	*L. innocua*
0	0	0	0	0
3	3.96	0.270 ± 0.165^{x}	$0.219 \pm 0.035^{x,y}$	$0.215 \pm 0.036^{x,y,z}$
5	6.61	0.372 ± 0.163^{x}	$0.414 \pm 0.049^{x,y}$	$0.380 \pm 0.027^{x,y,z}$
7	9.25	0.448 ± 0.161^{x}	$0.572 \pm 0.077^{x,y}$	$0.506 \pm 0.055^{x,y,z}$
10	13.22	0.522 ± 0.148^{x}	$0.687 \pm 0.077^{x,y}$	$0.596 \pm 0.052^{x,y,z}$
13	17.18	0.641 ± 0.154^{x}	$0.808 \pm 0.061^{x,y}$	$0.684 \pm 0.053^{x,y,z}$
17	22.47	0.739 ± 0.143^{x}	1.074 ± 0.079^{y}	0.825 ± 0.047^{y}
20	26.44	0.896 ± 0.136^{x}	1.403 ± 0.242^{y}	0.960 ± 0.054^{z}

$^{x, y, z}$Rows of means having a different letter are significantly different ($p<0.05$, time effect).

3. Result and discussion

3.1. Inactivation of E. coli K-12, E. coli O157:H7 and L. innocua in LEW samples

Influence of UV-C irradiation on *E. coli* K-12, *E. coli* O157:H7 and *L. innocua* inactivation in LEW at the UV intensity level of 1.314 mW/cm^2 and the sample depth of 0.153 cm is depicted in Fig. 2. The inactivation curve was constructed by plotting the log reduction (log (N/N$_0$)) versus exposure time (min). The populations of *E. coli* K-12, *E. coli* O157:H7 and *L. innocua* were reduced by 0.896, 1.403 and 0.960 logs respectively at a UV dose level of 26.44 mJ/cm^2 (UV dose was calculated from the applied exposure times and UV intensity) (Table 2). In other words, the highest log reduction (1.403-log CFU/ml) was achieved with pathogenic *E. coli* O157:H7 (ATCC 700728, NCTC 12900) after being exposed to UV-C irradiation for 20 min. According to these results, it was determined that this strain is less resistant to UV-C irradiation than that of non-pathogenic serotype strain (*E. coli* K-12) ($p<0.05$) under studied conditions. This finding is also supported by Hijnen et al. (2006). They determined *E. coli* O157:H7 to be more sensitive in water to UV irradiation compared to a non-pathogenic strain indicating that different *E. coli* strains exhibit different UV sensitivities.

Ngadi et al. (2003) applied 300 mJ/cm^2 (5 mW min/cm^2) UV dose at 0.315 mW/cm^2 incident UV intensity for 16 min in order to achieve 3.8-log (CFU/ml) reduction of *E. coli* O157:H7 (ATCC 35150) in LEW samples of 0.1 cm in depth. In their study, they calculated average UV

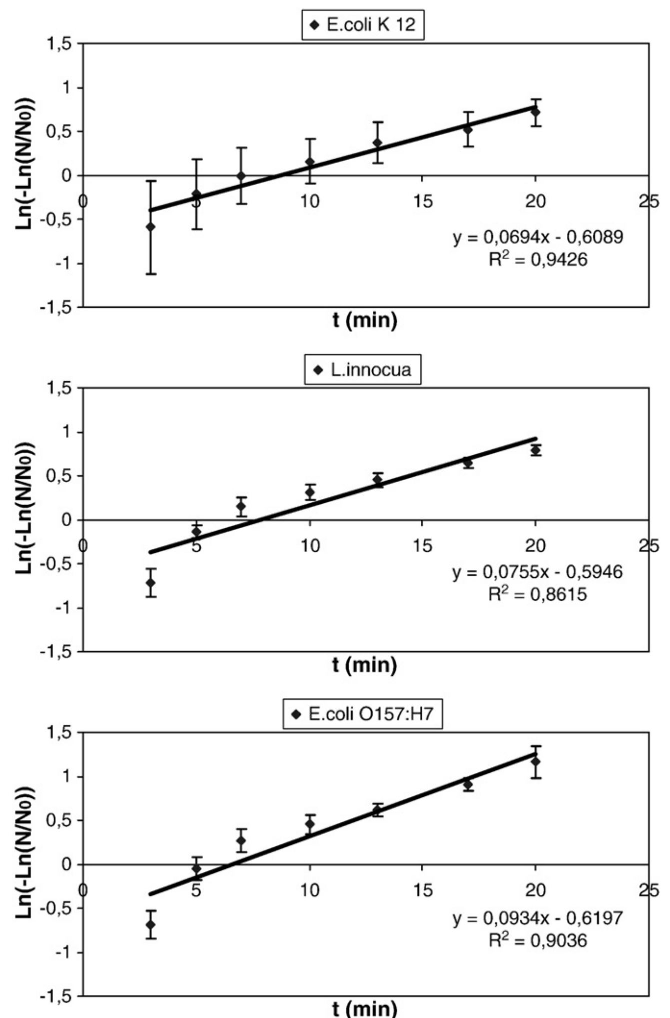

Fig. 3. The reasonable plots ln(−ln[N/N$_0$]) vs. ln(t) of the Weibull model.

Table 3
Weibull model parameters for *E. coli* K-12, *E. coli* O157:H7 and *L. innocua* using UV-C irradiation inactivation.

Microorganism	α_{ave} (min)	β_{ave}	t_{Rave} (min)[a]
E.coli K12	3.240 ± 1.649	0.716 ± 0.641	26.52 ± 25.98
E.coli O157:H7	2.003 ± 1.689	0.565 ± 0.361	20.29 ± 20.82
Listeria innocua	2.158 ± 1.886	0.708 ± 0.882	25.47 ± 21.60

[a] Based on average values of α and β, data showing high deviation are discarded while estimating t_R values.

intensity from Bouguer–Lambert law, which resulted in higher UV dose values than Beer–Lambert law that are used in this work. The variation in the log reduction values might be a result of differences in liquid depth, exposure time, *E. coli* strains, background flora and absorbance of LEW samples used in that study.

It was observed that Gram-positive bacteria *L. innocua* had greater resistance to UV-C exposure than that of *E. coli* O157:H7 in LEW. The differences in UV-C resistance of two bacterial types may arise from having different structural properties of cell walls. Gram-positive bacteria have thick peptidoglycan layers combined with periplasmic space and plasma membrane. UV-C light has little chance to penetrate across this rigid and thick cell layer. However, the gram-negative bacteria have only a single layer or thin multilayer of outer membrane (combination of lipopolysaccharide and protein), periplasmic space, peptidoglycan, and plasmic membrane (Blatchley et al., 2001). Geveke (2005) investigated the inactivation efficiency of UV-C irradiation on different gram-positive and negative bacteria in apple cider. *L. innocua* (NRRL-B 33314) was found to be less sensitive than *E. coli* to the UV treatment. In this study, it was observed that *L. innocua* and *E. coli* K-12 showed similar resistance to UV-C exposure. This is slightly different from those reported by Geveke (2005) for the inactivation of *E. coli* K-12 and *L. innocua* by UV-C irradiation. This difference may be related to the significant levels of indigenous microflora (>10^4) found in the LEW samples used for *L. innocua* experiments. The presence of a large number of background bacteria may cause shading and masking UV light to be absorbed by target microorganisms and thus leading to a lower level of

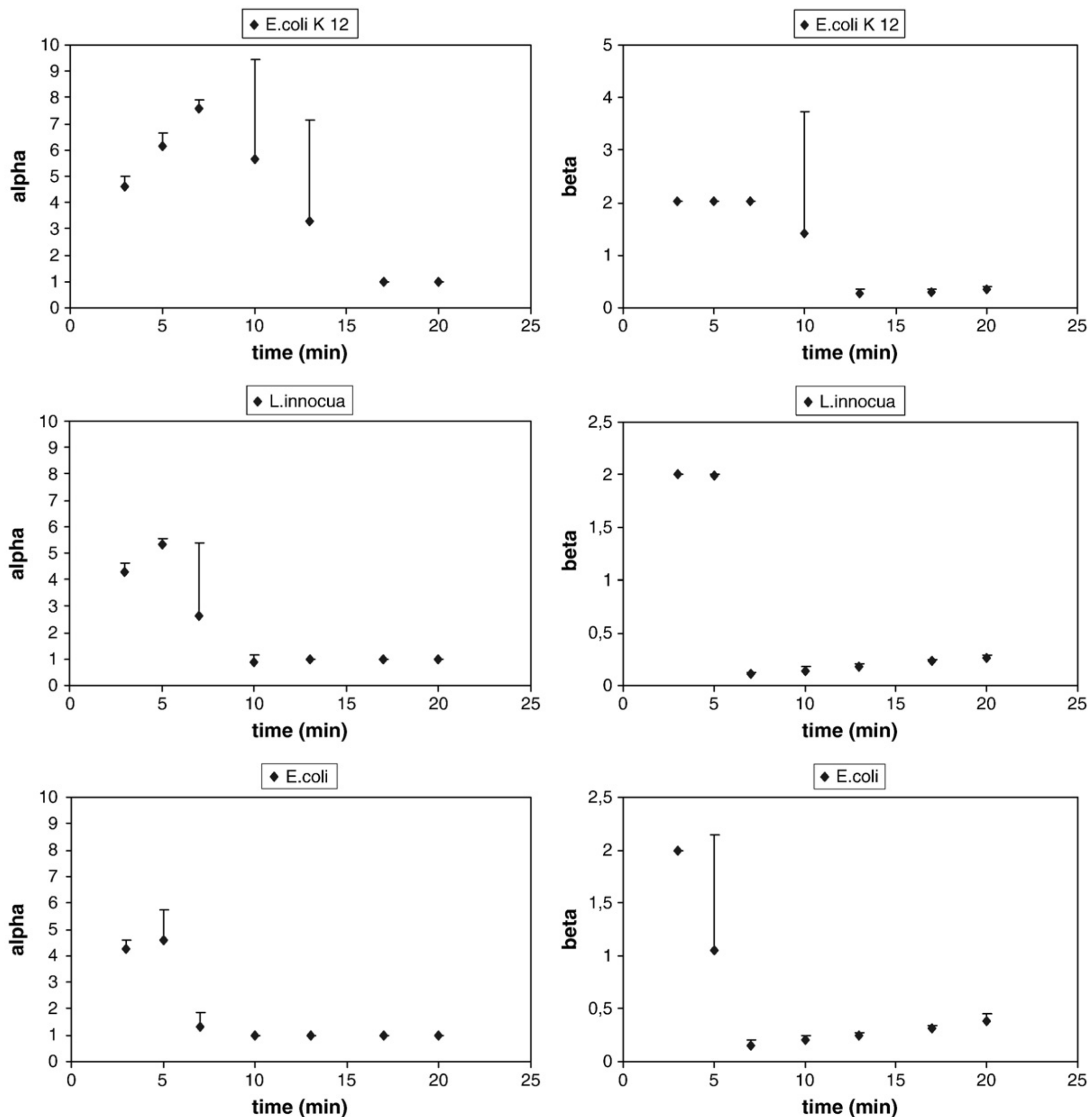

Fig. 4. Time dependency of Weibull model parameters.

Table 4
Hom model parameters for *E. coli* K-12, *E. coli* O157:H7 and *L. innocua* using UV-C irradiation inactivation.

Microorganism	k′ (min^{-1})	h
E.coli K12	0.068 ± 0.019	0.976 ± 0.014
E.coli O157:H7	0.073 ± 0.006	0.981 ± 0.014
Listeria innocua	0.065 ± 0.010	0.982 ± 0.015

Table 6
Modified Chick Watson model parameters for *E. coli* K-12, *E. coli* O157:H7 and *L. innocua* using UV-C irradiation inactivation.

Microorganism	k_1 (min^{-1})	k_2 (min^{-1})
E.coli K12	0.595 ± 0.221	0.990 ± 0.769
E.coli O157:H7	0.721 ± 0.389	0.940 ± 0.652
Listeria innocua	0.609 ± 0.267	0.936 ± 0.581

inactivation. As far as is known, there is no available literature on the inactivation of *L. innocua* in LEW by using UV-C irradiation with which a comparison can be made.

3.2. Modeling inactivation kinetics

The inactivation curves of microorganisms exposed to UV-C irradiation exhibited tailing and linear behavior, the shoulder was less pronounced (Fig. 2). Tailing can result from suspended material in the medium showing high turbidity that protects bacteria during irradiation, aggregates of cells caused by inhomogeneous distribution of microorganisms in the liquid medium and in-homogeneity in radiation (Unluturk et al., 2008). As a result, UV death kinetics were modeled by using both Log-Linear and non-Log-Linear (Weibull, Hom and modified Chick Watson) models by taking into account the tailing effect.

3.2.1. Weibull model

Applicability of Weibull model to experimental data was tested by plotting the double logarithms $\ln(-\ln(N/N_0))$ versus ln (t). This plot is known as a hazard plot (Nelson, 1972) in reliability engineering and must result in a straight line. Accordingly, reasonable plots were obtained and displayed in Fig. 3. Then the Weibull model was used to determine the tailing based on both the scale parameter (α) and the shape parameter (or concavity index) (β) (Table 3). For the three strains, β values were less than 1 indicating that remaining cells have less probability of dying or they become increasingly more resistant at the longer exposure times (van Boekel, 2002). The scale parameter α is usually considered as a measure of the organism resistance (kinetic parameter) to treatment and decreases with exposure time. α was found to be higher for *E. coli* K-12 than *L. innocua*. This was supported with $\log_{10}$ reduction values compared in Table 2. Lower reduction (0.896 log) was observed for *E. coli* K-12. Still it is difficult to withdraw a definite conclusion about the resistivity of these two microorganisms to UV-C irradiation. Consequently, the model predicted approximately similar t_R values (26.52 min for *E. coli* K-12 and 25.47 min for *L. innocua*). But this finding contradicts to the one reported in the previous section. This was attributed to the lack of clear shoulder and tailing part in the inactivation curves (Fig. 2) and the larger standard deviation observed in the experimental data. The larger standard deviation can be associated with the high background flora in LEW samples causing a competitive environment for *L. innocua* during UV-C exposure.

The dependence of shape parameter (α) and concavity index (β) on the exposure time was also examined (Fig. 4). α and β values obtained from inactivation of these three strains exhibited a more or less similar dependencies on exposure time. These findings were also supported with statistical analysis. The dependency of α and β on exposure time was statistically significant when tested at the 95% significance level (*$P<0.05$). It should be remarked that although there seemed to be a trend in exposure time dependency for these parameters as shown in Fig. 4, standard deviations for some α and β values were quite large and finding a linear relationship was difficult. The deviations may arise from the experimental artifacts such as fluctuation in UV light during exposure, in-homogeneity of liquid medium, clumping of microorganisms and sampling problems in spite of measures taken to avoid them.

3.2.2. Hom, Log-Linear and modified Chick Watson models

Three models, namely Hom, Log-Linear and modified Chick Watson; were used to describe the inactivation kinetics of *E. coli* K-12, *L. innocua* and *E. coli* O157:H7 exposed to UV-C irradiation treatment. All the parameters obtained from the fitting of these models to experimental measurements were shown in Tables 4, 5 and 6. The parameter h in the Hom model was found to be less than 1 for all the cases (Table 4). When $h<1$, Eq. (7) permits the fitting of a tail at the end of the UV-C treatment (Marugán et al., 2008). The second parameter in this model (k′) known as a kinetic constant gives an idea about the resistance of microorganisms to UV-C irradiation. According to this parameter, *L. innocua* was the most resistant and *E. coli* O157:H7 was the least resistant microorganism to UV-C irradiation. Geveke (2005) reached the same conclusion for *L. innocua* and *E coli*. Moreover, the non-pathogenic strain of *E. coli* (*E. coli* K-12) was found more resistant to UV light than the pathogenic strain (*E. coli* O157:H7) which is also supported by Hijnen et al. (2006). Although higher values of k were estimated from the Log-Linear model, the same conclusion can be drawn for *E. coli* K-12 and *E.coli* O157:H7 as in the case of the Hom model. Contrary, the linear model predicted a higher k value (0.095) for *L. innocua* than *E. coli* K-12 (0.08) (Table 5). The reason for this was pointed out in the previous section. D values were also calculated based on the first order kinetic inactivation. D value refers to the decimal reduction time that is equivalent to the number of minutes of exposure time to UV-C irradiation necessary to reduce the initial population of the test microorganisms by 1 $\log_{10}$ cycle. Based on the calculated D values per microorganism, *E. coli* O157:H7 was the least resistant to UV-C irradiation when suspended in LEW. Gabriel and Nakano (2009) reported that *E. coli* O157:H7 had lower resistance towards UV light compared to *E coli* K-12. This observation supports the results obtained

Table 5
Log-Linear model parameters for *E. coli* K-12, *E. coli* O157:H7 and *L. innocua* using UV-C irradiation inactivation.

Microorganism	k (min^{-1})	D (min)
E.coli K12	0.080	28.65
E.coli O157:H7	0.141	16.26
Listeria innocua	0.095	24.04

Table 7
Goodness of fit parameters for models.

Microorganism	Models	RMSE	R^2
E. coli K12	Linear	0.473	0.984
	Hom	0.150	0.980
	Chick Watson	0.066	0.920
	Weibull	0.202	0.830
E. coli O157:H7	Linear	0.783	0.933
	Hom	0.126	0.952
	Chick Watson	0.039	0.950
	Weibull	0.136	0.942
L. innocua	Linear	0.549	0.964
	Hom	0.094	0.961
	Chick Watson	0.039	0.981
	Weibull	0.071	0.981

from this study. They also showed that *L. monocytogenes* (M24-1) was more resistant strain compared to other test bacteria (*Salmonella typhimurium*, *Salmonella enteritidis*, *E.coli* O157:H7 and *E. coli* K-12) to UV light suspended in clear apple juice which contradicts to our results. The reason for that was explained in the previous sections.

Although the kinetic parameters (k_1 and k_2) estimated from the modified Chick Watson model (Table 6) were much higher (an order of magnitude higher) than Hom and Log-Linear models, the three models were in agreement that *E. coli* O157:H7 was the least resistant strain to UV-C irradiation under studied conditions.

The comparison of models was made based on RMSE and R^2, and presented in Table 7. modified Chick Watson model (2 parameter) provided a very good fit of the experimental data for three strains with the correlation coefficient (R^2) higher than 0.92. Although, R^2 values obtained from the other models were comparable with modified Chick Watson model, high RMSE values reduced the reliability of these models.

4. Conclusions

The efficiency of UV-C irradiation as a non-thermal pasteurization process for LEW was investigated. It was suggested that UV-C irradiation treatment could be used as a pretreatment process or alternative method when combined either with mild heat treatment or non-thermal technologies in order to reduce the initial microbial load and also adverse effects of thermal pasteurization of LEW. The inactivation rate in LEW was assessed by using non-pathogenic and pathogenic microorganisms. The inactivation kinetics of these tested microorganisms were best described by modified Chick Watson model (2 parameter) with the smallest RMSE ($R^2 \geq 0.92$). Although, R^2 values obtained from the Log-Linear model (1 parameter), Weibull model (2 parameters) and Hom model (2 parameters) were comparable with modified Chick Watson model, high RMSE values reduced the reliability of these three models.

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