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# Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light

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# Abstract

Inactivation data for *Escherichia coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries resulting from treatment with gaseous ozone, aqueous ozone, or pulsed UV-light were used to construct inactivation models; a log-linear model (based on first-order kinetics) and a Weibull model were developed. Initial analysis indicated that survival curves were non-linear and that the log-linear model failed to accurately estimate the inactivations in most instances. The Weibull model more accurately estimated the inactivation and the concavity exhibited in the survival curves. Validation of the Weibull model produced correlation coefficients of 0.83–0.99 and slopes of 0.76–1.26. The results presented in this study indicated that first-order kinetics are not suitable for the estimation of microbial inactivation on berries treated with ozone or pulsed UV-light, but that the Weibull model can be successfully used to estimate the reductions of *E. coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries.

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Keywords: Decontamination; Pathogens; Weibull; Small fruits; Novel technologies

#### 1. Introduction

Ozone and pulsed ultra violet (UV)-light are technologies that have been shown to have the potential for the decontamination of berries and other fresh food items (Bialka & Demirci, 2007a, 2007b; Kim & Yousef, 2000; Krishnamurthy, Demirci, & Irudayaraj, 2004; Sharma, Demirci, Beuchat, & Fett, 2002). However, to further understand the potential of these technologies the responses of microorganisms needs to be understood, through modeling. Traditionally, microbial inactivation has been described by first-order kinetics which assumes that under a constantly applied method of inactivation

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the number of viable cells will decrease exponentially and that all cells have the same probability of death. This assumes that a straight line will be derived from a semi-logarithmic plot. The frequent observance of shoulders, tails, and concavity in inactivation curves has led many to believe that the assumption of microbial inactivation as a first-order kinetic to be an exception rather than the norm (Peleg, 2006; Van Boekel, 2002).

The inactivation of *E. coli* in solution with ozone has been shown to follow first-order kinetics with respect to ozone concentration (Hunt & Marnias, 1997). And the inactivation of microorganisms via UV-light has also been shown to follow first-order kinetics in solution (EPA, 2003), but has also been reported to display a sigmoidal shape with a shoulder and/or a tail (CFSAN-FDA, 2006).

The Weibull distribution is being used to a greater extent to describe microbial inactivation, and is based on the engineering principle of failure. Instead of a structural

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or mechanical failure, the failure is that of the microorganism (Peleg, 2006). This model has been used to describe the heat inactivation of L. monocytogenes, S. Enteritidis, S. Typhimurium, E. coli O157:H7 and Staphylococcus aureus, which exhibited a tailing concave survival curves (Buzrul & Alpas, 2007). This model has been successfully used to model other novel processing technologies. The inactivation of Staphylococcus aureus during high pressure processing was successfully modeled using a Weibull distribution (Guan, Chen, Ting, & Hoover, 2006). The inactivation of *Listeria innocua* with pulsed electric fields was successfully estimated using the Weibull distribution (San Martin et al., 2007). The authors also pointed out that the estimates obtained using the Weibull distribution were better than those estimates obtained using models based on first-order kinetics.

The goal of this study was to model the inactivation of *E. coli* O157:H7 and *Salmonella* on raspberries and strawberries resulting from treatments with aqueous ozone, gaseous ozone, or pulsed UV-light.

#### 2. Materials and methods

## 2.1. Preparation of inoculum

Five strains of nalidixic acid resistant E. coli O157:H7 and Salmonella enterica were obtained from the Center for Food Safety at the University of Georgia. The E. coli O157:H7 strains were: 932 (human isolate), 994 (salami isolate), E0018 (calf fecal isolate), H1730 (human isolate from outbreak associated with lettuce), and F4546 (human isolate from outbreak associated with alfalfa sprouts). The Salmonella enterica serotypes used were: Agona (human isolate from outbreak associated with alfalfa sprouts), Baildon (human isolate from outbreak associated with diced tomatoes), Gaminara (orange juice isolate), Michigan (human isolate associated with cantaloupe outbreak), and Montevideo (human isolate associated with tomato outbreak). Cultures were grown in tryptic soy broth (Becton Dickinson, Sparks MD) supplemented with 50 µg/ml nalidixic acid (Fisher Scientific Co., Fair Lawn, NJ) at 37 °C for 24 h. A mixture of E. coli O157:H7 or Salmonella strains were prepared by combining 10 ml of each culture and centrifuging for 15 min at 3300g and 4 °C. The supernatant was discarded and the cells were resuspended in 10 ml of 0.1% peptone water (Becton Dickison) to yield an approximate population of  $10^8$  CFU/ml.

#### 2.2. Inoculation of berries

Red raspberries and strawberries were purchased from a local grocery store and left at room temperature for 1 h prior to inoculation. To inoculate the raspberries, a 25  $\mu$ L droplet of inoculum was deposited on the skin of each fruit. For strawberries, a 50  $\mu$ L droplet of inoculum was deposited on the skin approximately midway between the calyx and cap (Yu, Newman, Archbold, & Hamilton-

Kemp, 2001). The fruits were dried in a laminar flow hood for 24 h before the treatment to allow for attachment of the microorganisms. The inoculated raspberries and strawberries had approximately  $10^5$  CFU/g fruit of either *E. coli* O157:H7 or *Salmonella*.

# 2.3. Treatments with gaseous or aqueous ozone

Both the gaseous and aqueous ozone treatments of raspberries and strawberries were carried out as described in Bialka and Demirci (2007a) and Bialka and Demirci (2007b), respectively.

## 2.4. Treatment with pulsed UV-light

Pulsed light (100-1100 nm) was produced using a laboratory scale, batch-fed pulsed-light system (Steripulse-XL 3000, Xenon Corp., Wilmington, MA). The system generated 1.27 J/cm<sup>2</sup> per pulse for an input of 3,800 V and with 3 pulses per second at 1.8 cm from the quartz window per the manufacturer's specifications. The distance between the UV-strobe and the quartz window is 5.8 cm. Fruits were treated at three different distances from the quartz window measured from the bottom of the fruit; 3 cm, 8 cm, and 13 cm for raspberries and 5 cm, 8 cm, and 13 cm for strawberries. For strawberries a distance of 5 cm was used instead of 3 cm due to their larger size. Furthermore, the surface of the fruit which was inoculated was oriented toward the light so that it could receive full exposure. At each distance from the quartz window, 5, 10, 30, 45, and 60 s treatment times were evaluated. The amount of broad band energy the fruit received was measured using a Nova Laser Power energy monitor (Ophir Optronic Ltd., Wilmington MA) which averaged the energy across 30 pulses with the face of the sensor located at the same height as the bottom the fruits. Temperature of the fruit was monitored using a K-type thermocouple (Omegaette HH306, Omega Engineering, Inc., Stamford, CT) by placing the thermocouple 1-2 mm under the surface of the fruit. The maximum temperatures achieved were 80 and 45 °C for raspberries and strawberries, respectively, after 60 s of treatment at the distance deemed "best".

The treatment which resulted in the maximum  $log_{10}$  reductions and minimum fruit damage was chosen for the model development. For raspberries, the treatment level was selected as 3 cm from the quartz window which had broadband energy (wavelengths between 100 and 1100 nm, with 54% of the energy attributed to UV-light as per the manufacturer) doses between 6 and 72 J/cm<sup>2</sup> and for strawberries the level was 13 cm with doses between 5.4 and 64.4 J/cm<sup>2</sup>.

#### 2.5. Microbial analysis

After treatment, strawberries were placed in 100 ml of Dey-Engley Neutralizing (D/E) Broth (Difco) and raspberries were placed in 50 ml D/E broth and pummeled for

l min in a stomacher. The homogenate was then serially diluted in 0.1% peptone water (Becton Dickinson) and spiral plated on tryptic soy agar (Becton Dickinson) supplemented with 50  $\mu$ g/ml of nalidixic acid using an Autoplate 4000 (Spiral Biotech, Norwood, MA). Plates were incubated at 37 °C for 24 h and then enumerated using Q-count (Version 2.1, Spiral Biotech, Norwood, MA). Reductions of bacteria were calculated on a per gram of fruit basis.

## 2.6. Model development

Traditionally, microbial inactivation has been described by first-order inactivation kinetics (Eq. (1)) which was termed the log-linear equation.

$$\log_{10}\left(\frac{N}{N_0}\right) = -kt \tag{1}$$

where N the number of microorganisms at time t (CFU/g),  $N_0$  initial number of microorganisms (CFU/g), t treatment time (s or min), k first-order extinction coefficient (s<sup>-1</sup> or min<sup>-1</sup>).

From this equation the classic "*D*-value" or the time necessary for a  $1 \log_{10}$  reduction can be determined and is calculated as the reciprocal of the first-order rate constant. The log-linear equation is only appropriate for linear inactivation curves (where time is on the *x*-axis and  $\log_{10} (N/N_0)$  is on the *y*-axis) and research has found that many inactivation curves are non-linear.

The second model used in the study is the Weibull model (Eq. (2)), which has been historically used in failure engineering (Van Boekel, 2002).

$$\log_{10}\left(\frac{N}{N_0}\right) = -\frac{1}{2.303} \left(\frac{t}{\alpha}\right)^{\beta} \tag{2}$$

where N the number of microorganisms (CFU/g),  $N_0$  initial number of microorganisms (CFU/g), t treatment time (s or min),  $\alpha$  characteristic time (s or min),  $\beta$  shape parameter (unitless).

Many survival curves exhibit concavity, either downwards or upwards, and the  $\beta$  parameter is used to describe this concavity. If  $\beta < 1$  the curve displays upward concavity and if  $\beta > 1$  the curve displays downward concavity. Also, the parameters  $\alpha$  and  $\beta$  can be used to calculate the reliable life,  $t_{\rm R}$ , or the 90% percentile of the failure time distribution (Van Boekel, 2002), which is analogous to the *D*-value for the first log reduction (Eq. (3)).

$$t_R = \alpha * (2.303)^{\frac{1}{\beta}} \tag{3}$$

where  $\alpha$  characteristic time,  $\beta$  shape parameter.

The models were constructed using averages from two experimental data sets, and a third data set was used for validation. To achieve a best-fit, parameter estimation was performed by minimizing the sum of square error between the experimental and estimated  $log_{10}$  reductions, using non-linear least squares regression method in Microsoft Excel 2000. Validation of the model was conducted by

back-predicting the third experimental data set and performing a linear regression with the estimated versus the experimental data.

#### 3. Results and discussion

The reductions of *E. coli* O157:H7 and *Salmonella* on raspberries and strawberries after treatment with gaseous and aqueous ozone or pulsed UV-light was evaluated. Models were developed to estimate the reduction of *E. coli* O157:H7 and *Salmonella* in terms of ozone exposure time or energy dose, using both log-linear and Weibull assumptions.

#### 3.1. Aqueous ozone models

The fraction of survivors  $\left(S = \frac{N}{N_0}\right)$  after treatment with aqueous ozone a similar trend was observed; which is the tailing off of survivors (Bialka & Demirci, 2007a). For the log-linear model, two sets of data were fit using linear regression and the root mean square error (RMSE) was used as a measure of goodness-of-fit (Table 1). To validate the model,  $\log_{10} (S)$  values were back predicted with the third data set and a linear regression was performed for estimated versus experimental values to determine the correlation coefficient ( $R^2$ ) and slope. For *E. coli* O157:H7 the RMSE values were 0.26 and 0.18 for raspberries and strawberries, respectively; and for Salmonella the RMSE values were 0.09 and 0.19 for raspberries and strawberries, respectively. The failure of the model to accurately estimate  $\log_{10}$ (S) values can be seen in the correlation coefficients of 0.56 and 0.62 and slopes of 0.51 and 0.54 for raspberries and strawberries, respectively, for estimations of E. coli O157:H7 inactivations. For Salmonella, correlation coefficients were 0.66 and 0.86 and slopes were 0.53 and 0.77 for raspberries and strawberries, respectively. It was concluded that these slopes, RMSEs and correlation coefficients were not acceptable.

The ability of the Weibull model to more accurately estimate the values of  $\log_{10} (S)$  can be seen in Table 1 were RMSE values for *E. coli* O157:H7 were 0.09 and 0.07 for raspberries and strawberries, respectively and are less than those for the log-linear model. Also, the correlation coeffi-

Table 1

Goodness-of-fit parameters of two models estimating reductions of *E. coli* O157:H7 and *Salmonella* on raspberries and strawberries after treatment with aqueous ozone

| Fruit      | Microorganism          | Models                | RMSE         | $R^2$        | Slope        |
|------------|------------------------|-----------------------|--------------|--------------|--------------|
| Raspberry  | <i>E. coli</i> O157:H7 | Log-linear<br>Weibull | 0.26<br>0.09 | 0.56<br>0.95 | 0.51<br>0.85 |
|            | Salmonella             | Log-linear<br>Weibull | 0.09<br>0.17 | 0.66<br>0.88 | 0.53<br>0.76 |
| Strawberry | <i>E. coli</i> O157:H7 | Log-linear<br>Weibull | 0.18<br>0.07 | 0.62<br>0.99 | 0.54<br>0.97 |
|            | Salmonella             | Log-linear<br>Weibull | 0.19<br>0.12 | 0.86<br>0.98 | 0.77<br>1.06 |

cients were much higher at 0.95 and 0.99 for raspberries and strawberries, respectively. The goodness-of-fit parameters obtained for the inactivation of *Salmonella* on raspberries and strawberries were 0.17 and 0.12 for RMSE values and 0.88 and 0.98 for the correlation coefficient. The fit of the Weibull model to the experimental data can be seen in Fig. 1, for *E. coli* O157:H7 and *Salmonella*, respectively.

The Weibull model consists of two parameters, which are presented in Table 2, the  $\alpha$  value corresponds to the mean of the distribution which describes death times, and can be used to determine a failure time or the time in which a 1 log<sub>10</sub> reduction will occur. Overall, reductions of *E. coli* O157:H7 occur faster than reductions of *Salmonella*, with  $t_{\rm R}$  values of 0.13 and 1.49 min for raspberries and strawberries, respectively; whereas  $t_{\rm R}$  values for *Salmonella* were 2.39 and 2.60 for raspberries and strawberries. The  $\beta$ parameters, for both *E. coli* O157:H7 and *Salmonella* are all less than 1 and indicates that the remaining cells have a reduced probability of dying or being reached by the ozone.

#### 3.2. Gaseous ozone models

The inactivation curves of *E. coli* O157:H7 and *Salmo-nella* after treatment with gaseous ozone can be obtained from Bialka and Demirci (2007b). It should be noted that



Fig. 1. Fit of Weibull model to aqueous ozone inactivation data.

Table 2 Weibull model parameters for reductions of *E. coli* O157:H7 and *Salmonella* using aqueous ozone

| Fruit      | Microorganism   | α     | β    | $t_{\rm R}$ (min) |
|------------|-----------------|-------|------|-------------------|
| Raspberry  | E. coli O157:H7 | 0.007 | 0.29 | 0.13              |
|            | Salmonella      | 0.42  | 0.48 | 2.39              |
| Strawberry | E. coli O157:H7 | 0.06  | 0.27 | 1.49              |
|            | Salmonella      | 0.32  | 0.40 | 2.60              |

reductions of *Salmonella* on strawberries could not be fitted using either model. As with the inactivation curves observed for aqueous ozone there is a tailing effect as the treatment time approached 64 min. The Weibull model provided better estimations of microbial inactivation (Fig. 2) after treatment based on RMSE, slope, and  $R^2$  values (Table 3). For *E. coli* O157:H7 these values were 0.06, 1.15, and 0.99 for raspberries, respectively; and 0.06, 1.05, and 0.99 for strawberries, respectively. For reductions of *Salmonella*, the RMSE, slope and  $R^2$  values were 0.09, 0.86, and 0.98, respectively, on raspberries.

As with the reductions obtained after aqueous ozone treatment the  $\beta$  value is less than 1, which indicates that the majority of the remaining cells are resistant to the treatment (Table 4). Like for the aqueous ozone treatments, the  $\alpha$  values for this treatment exhibit no trend based on the microorganism.

Also, the time necessary to achieve a  $\log_{10}$  reduction is much greater for the gaseous ozone treatments than the aqueous ozone treatments, with a maximum  $t_{\rm R}$  of 12.8 min for gaseous ozone versus 2.6 min for aqueous ozone. Overall, the Weibull model fits the data obtained after treatment with gaseous ozone giving RMSE values less than those for the log-linear model, and larger correlation coefficients after model validation.



Fig. 2. Fit of Weibull model to gaseous ozone inactivation data.

Table 3

Goodness-of-fit parameters of two models estimating reductions of *E. coli* O157:H7 and *Salmonella* on raspberries and strawberries after treatment with gaseous ozone

| Fruit      | Microorganism   | Models     | RMSE            | $R^2$ | Slope |
|------------|-----------------|------------|-----------------|-------|-------|
| Raspberry  | E. coli O157:H7 | Log-linear | 0.09            | 0.98  | 0.98  |
|            |                 | Weibull    | 0.06            | 0.99  | 1.15  |
|            | Salmonella      | Log-linear | 0.16            | 0.70  | 0.53  |
|            |                 | Weibull    | 0.09            | 0.98  | 0.86  |
| Strawberry | E. coli O157:H7 | Log-linear | 0.12            | 0.90  | 0.77  |
|            |                 | Weibull    | 0.06            | 0.99  | 1.05  |
|            | Salmonella      | Log-linear | NA <sup>a</sup> | NA    | NA    |
|            |                 | Weibull    | NA              | NA    | NA    |

<sup>a</sup> Not applicable (neither model could be used to obtain estimates).

| Sumerica using gaseous choire |                 |                 |      |                |  |
|-------------------------------|-----------------|-----------------|------|----------------|--|
| Fruit                         | Microorganism   | α               | β    | t <sub>R</sub> |  |
| Raspberry                     | E. coli O157:H7 | 3.27            | 0.61 | 12.81          |  |
|                               | Salmonella      | 0.33            | 0.28 | 6.60           |  |
| Strawberry                    | E. coli O157:H7 | 0.95            | 0.40 | 7.59           |  |
|                               | Salmonella      | NA <sup>a</sup> | NA   | NA             |  |

Table 4 Weibull model parameters for reductions of *E. coli* O157:H7 and *Salmonella* using gaseous ozone

<sup>a</sup> Not applicable.

## 3.3. Pulsed UV-light models

Models describing the inactivation of *E. coli* O157:H7 and *Salmonella* on raspberries and strawberries were developed based on the treatment level that produced the highest  $\log_{10}$  reductions and resulted in minimal damage to the fruit. The microbial survival curves can be seen in Fig. 3, where several shapes are obtained depending on fruit and microorganism combination. Two models were compared, the log-linear and Weibull; the goodness-of-fit parameters can be seen in Table 5. Overall, the RMSE and  $R^2$  values obtained for the Weibull model are better than those obtained for the log-linear model. From the inactivation curves, it can easily be observed that none of the berrymicroorganism combinations exhibit a linear trend.

For E. coli O157:H7 the RMSE values obtained using the Weibull model were 0.23 and 0.06 for raspberries and strawberries, respectively, with correlation coefficients of 0.91 and 0.98, respectively. The ability of this model to accurately estimate reductions of E. coli O157:H7 can be seen in Fig. 4. The shape of the survival curves is different for each berry, with strawberry exhibiting a tailing effect. The Weibull model parameters (Table 6) provide more insight into the shapes of these inactivation curves. For strawberries, the  $\beta$  parameter is less than 1, which accounts for its upward concavity, but also indicating that the remaining cells less susceptible to pulsed UV-light, perhaps because they are being shielded by the achnes of the strawberry or are in between the druplets of the raspberries. The opposite can be observed for the inactivation of E. coli O157:H7 on raspberries; which display a downward concavity, with a  $\beta$  parameter greater than 1, which may be due to a different distribution of cells on the fruit.

A trend can also be seen in the dose required for  $1 \log_{10}$  reduction  $(d_R)$ , with  $d_R$  values much less for strawberries indicating that the most susceptible cells are inactivated quickly, but much larger  $d_R$  values are observed on raspberries illustrating the cumulative effect of the treatment. Reductions of *Salmonella* exhibit a similar trend for the fit of the Weibull model, except that none of the curves exhibit downward concavity (Fig. 4). All berries had  $\beta$  values less than 1, which probably indicates that susceptible



Fig. 3. Influence of broadband energy dose on microbial reduction after treatment with pulsed UV-light on raspberries and strawberries.

Table 5

Goodness-of-fit parameters of two models estimating reductions of *E. coli* O157:H7 and *Salmonella* on raspberries and strawberries after treatment with pulsed UV-light

| Fruit      | Microorganism   | Models     | RMSE | $R^2$ | Slope |
|------------|-----------------|------------|------|-------|-------|
| Raspberry  | E. coli O157:H7 | Log-linear | 0.57 | 0.80  | 1.11  |
|            |                 | Weibull    | 0.23 | 0.91  | 1.08  |
|            | Salmonella      | Log-linear | 0.31 | 0.50  | 0.28  |
|            |                 | Weibull    | 0.06 | 0.92  | 0.93  |
| Strawberry | E. coli O157:H7 | Log-linear | 0.06 | 0.69  | 0.56  |
|            |                 | Weibull    | 0.06 | 0.98  | 0.90  |
|            | Salmonella      | Log-linear | 0.21 | 0.86  | 0.87  |
|            |                 | Weibull    | 0.02 | 0.96  | 1.20  |



Fig. 4. Fit of Weibull model to pulsed UV-light inactivation data.

Table 6 Weibull model parameters for reductions of *E. coli* O157:H7 and *Salmonella* using pulsed UV-light

| Sumonena asing paised of fight |                 |       |      |             |  |
|--------------------------------|-----------------|-------|------|-------------|--|
| Fruit                          | Microorganism   | α     | β    | $d_{\rm R}$ |  |
| Raspberry                      | E. coli O157:H7 | 20.5  | 1.87 | 32.10       |  |
|                                | Salmonella      | 4.16  | 0.71 | 13.33       |  |
| Strawberry                     | E. coli O157:H7 | 0.009 | 0.23 | 0.32        |  |
|                                | Salmonella      | 0.05  | 0.32 | 0.69        |  |

populations have been inactivated and the remaining are trapped within the crevices of the fruit are unable to be inactivated.

As indicated by the results, the Weibull model was found to much more accurately estimate the microbial reductions obtained during gaseous ozone, aqueous ozone, and pulsed UV-light treatments. Koseki and Yamamoto (2007) found that the Weibull model as well as the modified Baranyi model could be used to accurately estimate the reductions of *E. coli* during high pressure processing. The authors also found that a linear model was not suitable to describe these inactivations. The Weibull and modified Baranyi models were capable of fitting the tail of the survivor curve as was observed in this study. Klotz, Pyle, and Mackey (2007) also noted that traditional models were not capable of fitting the tails and concavity encountered in survival curves resulting from high pressure processing.

# 4. Conclusion

The results presented here further illustrate the misconception that reductions of microorganisms within or on a food exhibit a log-linear trend. This was expected for both the aqueous ozone treatment and the pulsed UV treatment since the concentration or temperature, respectively, was increasing with time; however, with the gaseous ozone treatment the concentration was constant which is the general assumption used in a log-linear model, and as the data shows the Weibull model produces better estimations of microbial reduction than the log-linear model. As noted by Van Boekel (2002) the majority of inactivation curves exhibit concavity. Only 1 out of 11 models approached a first-order kinetic with  $\beta$  values approaching 1. Van Boekel associates the concavity in the curves to an adaptation of the microorganisms to the treatment; within this research much of the "adaptation" may be attributable to the location of the microorganisms on the fruit. Microorganisms may have been shielded from the treatment by being located with the druplets of the raspberries or under the achnes of the strawberries. In order to estimate the reductions of E. coli O157:H7 and Salmonella on raspberries and strawberries after treatment with ozone or pulsed UV-light, the Weibull model was successfully used, since it consistently and accurately estimated reductions of all fruits, treatment, and microorganisms. Further research into the effect of the physical structure of the food on microbial inactivation resulting from ozone and pulsed UV-light is needed.

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