Comparative analyses of prediction models for inactivation of *Escherichia coli* in carrot juice by means of pulsed electric fields

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Abstract This paper reports the prediction capacity of various microbial inactivation models to prefigure the bactericidal effect using pulsed electric field (PEF) on liquid food. The aim of study was to compare the various inactivation models based on accuracy and bias factor to find out the most accurate inactivation model for *Escherichia coli* present in carrot juice treated with PEF. In this study, *E. coli* suspended in carrot juice was treated with varying pulsed electric field strength for different intervals. The obtained data were utilized for the evaluation of parameters of Bigelow, Peleg, Hülshager and Weibull inactivation models. Furthermore, secondary models were developed for Hülshager and Weibull to predict the microbial inactivation at any level of field strength and treatment time. The secondary model for Hülshager exhibits 5.8% error as compared to the Weibull model having 8.5% error in prediction of death kinetics of *E. coli* suspended in carrot juice by means of PEF. The comparative analysis of secondary models to forecast the unknown data set unveiled the superior functioning of Hülshager model.

Keywords Microbial inactivation models · Bias factor · Accuracy factor · Field strength · Treatment time

Introduction

Thermal treatments techniques, are traditional methods of pasteurization, which encounter the problem of degradation of sensory and nutritional attributes of the food (Jayaram et al. 1992). However, they are still most commonly used pasteurization methods due to unavailability of commercial viable non-thermal pasteurization techniques. Recently many non-thermal preservation technologies have been proposed. Among all emerging non-thermal technologies, high intensity PEF processes the food without compromising on taste and flavor (Lado and Yousef 2002; Abram et al. 2003). For example, the PEF treatment to carrot juice assured insignificant diminution in pH, electric conductivity, color, brix and titratable acidity along with log reduction of 4.3 and 3.4 *D* for mold and yeast respectively while keeping the mineral element persevered (Akin and Evrendilek 2009). The physicochemical properties of PEF treated carrot juice such as total acidity, color and viscosity are equivalent to that of fresh juice (Xiang et al. 2014). All these facts elucidate that an effective PEF treatment system can meet the consumers demands. Whereas, carrot juice treated with 110 °C for 30 s lessened α carotene from 27.6 to 15.0 $\mu\text{g/ml}$ and β -carotene from 62.5 to 34.4 $\mu\text{g/ml}$ and also shows significant deprivation in color (Chen et al. 1995). Thus, need of non-thermal technology for carrot juice is pious and just.

To design an effective PEF pasteurization treatment, accurate mathematical prediction models are primarily required (Álvarez et al. 2003). These models colligate the various PEF treatment parameters to survival fractions in order to estimate the inactivation level (Hülshager et al. 1981; Peleg 1995; Peleg and Cole 1998; Van Boekel 2002). Usually for the reduction of few $\log_{10}$ cycles, the survival curves follow the first order log-linear relationship. For the

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instances where the survival curves may not follow the log-linear relationship, alternative mathematical models are desirable to describe the survival curves (Rivas et al. 2006). Immense studies have been carried out in buffered media (Aronsson et al. 2005; Toepfl et al. 2007) and actual food (Saldaña et al. 2011) to canvass the inactivation kinetics of pathogen and yeast. Various models based on the empirical and phenomenological considerations have been proposed (Peleg and Pechina 2000; Van Boekel 2002). As all these models are based on certain supposals, therefore, the accuracy of model is always incertitude. One model can describe survival curves accurately for a given pathogen in a peculiar food, but it may fail for other food. Hence, different models must be compared in terms of their performances to find out which model predicts inactivation accurately. Few numbers of such comparative studies have been reported but all of them are in buffered or model food medium. No study has been reported on comparative analysis of predication models for pure carrot juice sample.

In this work, carrot juice inoculated with *E. coli* was treated with PEF and ascertained data is fitted on various inactivation models. Four models were selected to describe the survival curve fraction of *E. coli* and their predicting capacities are compared based on the accuracy factor, bias factor, and mean square error. Two models with better accuracy were used to develop secondary models. The developed secondary models were validated on the unknown dataset, which was not used for the generation of model.

Material and methods

Preparation of carrot juice

The carrots were purchased from the local market and washed properly with tap water then peeled and cut into small slices and pressed in the juicer. The juice obtained was filtered with mesh filter and centrifuged at 12000 rpm for 15 min in order to get clarified juice. The conductivity and pH of juice were 6.0 mS/cm and 6.03 respectively. The carrot juice was diluted to 3 times for the safe running of operation in SAMTECH PEF integrated system (Glasgow, UK).

Microbial growth

The lyophilized strains of *E. coli* (MTCC 1687) used in the study were obtained from the Institute of Microbial Technology (CSIR-IMTECH) Chandigarh, India. These strains were rehydrated and grown on nutrient agar¹ media. The

loop full of harvested microorganisms were transferred from solid to liquid form by inoculating them into sterilized nutrient broth followed by incubation period of 24 h at 37 °C. The final population in carrot juice was adjusted to 30×10^6 cfu/ml.

Bacterial enumerations

The bacterial enumerations were figured with plate count method. The colonies are grown on the nutrient and EMB agar² by spreading the diluted control and treated samples. The saline water (0.85% of NaCl) was used for serial dilution and results are recorded in triplicate. The mean average value of all the recorded results were taken as final approximate of the bacterial population.

Experimental

This study was carried out with an integrated PEF treatment system by SAMTECH (Glasgow, UK). This integrated system consists of generator and a static parallel plate treatment chamber of volume 5 ml. During the course of experimentation, in order to avoid contamination, the treatment chamber was rinsed thoroughly with ethanol followed by autoclaving and then filled manually with carrot juice. This procedure was repeated before each experiment. Precautions were taken to avoid air bubbles in the treatment chamber which often arises due to uneven filling of the sample. The square unipolar pulses with negative polarity and fixed pulse width of 1 μ s at 1 Hz frequency had been utilized.

The strength of voltage signal across two plates was measured with the Tektronix digital oscilloscope (TDS1001B) via high voltage probe (P6015A). Pathogen *E. coli* was suspended in carrot juice and microbial population was adjusted to 10^6 cfu/ml. The conductivity (σ) of inoculated juice was adjusted to 3.78 mS/cm. The variation in conductivity of carrot juice was scrutinized with fluctuation in temperature and it was found that the conductivity of carrot juice was below 4 mS/cm (desired level for 5 ml treatment chamber of SAMTECH integrated PEF system) up to 60 °C.

Mathematical prediction models

To establish the relationship between the microbial inactivation and the field strength, researchers have proposed different models based on empirical and phenomenological consideration (Álvarez et al. 2003). Following inactivation

¹ With ingredients per liter: Peptic digest of animal tissue 5 gm, Sodium chloride 5 gm, Beef extract 1.5 gm, Yeast extract 1.5 gm, and Agar 15 gm with final pH (at 25 °C) 7.4 ± 0.2 .

² With ingredients per liter: Pancreatic Digest of Gelatin 10 gm, Lactose 10 gm, Dipotassium Phosphate 2 gm, Eosin Y 0.4 gm, Methylene Blue 65 mg and Agar 15 gm.

models have been selected because of ease of their use to study the inactivation kinetics of *E. coli* in model as well as actual food samples.

Model 1 (Bigelow model)

Bigelow model (Bigelow 1921) relates the survival fraction to treatment time with a first-order equation as

$$\log(S) = -\frac{t}{D} \quad (1)$$

where, S is survival fraction

$$S = \frac{N_t}{N_o} \quad (2)$$

N_t is number of colony forming unit (cfu) after the treatment and N_o is number of cfu before the treatment.

Model 2 (Peleg model)

Survival curves for some microorganisms show sigmoid nature after PEF treatment. Based on these types of variations, Peleg proposed a model for microbial inactivation, known as Peleg Model (Peleg 1995; Peleg and Cole 1998) which represents the field strength and survival fraction as

$$S = \frac{100}{1 + e^{\frac{E-E_c}{k}}} \quad (3)$$

here, E is the applied field strength, E_c is critical field strength and k is parameter indicating the steepness of survival curve around E_c . Critical field intensity can be calculated by extrapolating the survival fraction for 50% survival as

$$E_c = E - k \ln\left(\frac{1-S}{S}\right). \quad (4)$$

Model 3 (Hülshager model)

Hülshager et al. (1981) proposed a model based on the empirical consideration that depicts the natural log reduction to field strength given by

$$\ln(S) = -b_E \cdot (E - E_c) \quad (5)$$

where, E is the applied field strength (kV/cm), b_E is coefficient of regression (cm/kV), E_c is critical field strength, required for the inactivation and can be calculated as

$$E_c = 1 - \frac{\ln(S)}{b_E} \quad (6)$$

Critical field strength belongs to 100% survival and can be calculated by extrapolating the value of E for unity survival ratio.

Model 4 (Weibull distribution model)

This model is a mathematical equation based on Weibull distribution function (Peleg and Cole 1998; Van Boekel 2002) which describes the survival curves non-logarithmically as

$$\log_{10} S = -\left(\frac{t}{a}\right)^n \quad (7)$$

here, t is treatment time, n and a are the parameters of shape and scale. The shape parameter ' n ' defines shape of survival curve, when $n = 1$, this model is equivalent to Bigelow model, given in Eq. 1.

Statistical analysis

The performance indices of predicting models are accessed with accuracy factor (Af) and bias factor (Bf) (Ross 1996) given by

$$Af = 10 \left(\sum \frac{\left| \log \left(\frac{\text{predicted}}{\text{observed}} \right) \right|}{n} \right) \quad (8)$$

$$Bf = 10 \left(\sum \frac{\log \left(\frac{\text{predicted}}{\text{observed}} \right)}{n} \right) \quad (9)$$

$Af = 1$ indicates the predication with zero error, Bf indicates nature of fit weather over fit, under fit or perfect fit. The fitting accuracy of models are indicated with mean square error (MSE) and coefficient determination (R^2) defined as

$$MSE = \frac{\sum (\text{Predicted} - \text{Observed})^2}{n} \quad (10)$$

' n ' is number of observation.

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} \quad (11)$$

where SS_{res} and SS_{tot} are sum of squares of residuals and total sum of squares calculated from

$$SS_{tot} = \sum_i (y_i - \bar{y})^2 \quad (12)$$

$$SS_{res} = \sum_i (f_i - y_i)^2 \quad (13)$$

y_i are the observed values and $\bar{y}$ is the mean value of observed data and f_i are modeled values. Accuracy factor equal to 1 indicates the condition of perfect fit. Coefficient of determination indicates the agreement between the

Fig. 1 Effect of field strength and treatment time on *E. coli* with pulsed electric field

predicted and observed values of data $R^2 = 1$, is the condition of perfect fit.

Results and discussion

The $\log_{10}$ cycle of *E. coli* inactivation as an effect of field strength and treatment time of 9–21 kV/cm and 100–500 μ s respectively, is shown in Fig. 1. The negative sign indicates the reduction in bacterial population in both nutrient and EMB agar mediums. Increase in the inactivation level of *E. coli* was observed with increase in treatment time and field strength (Fig. 1). At a particular field strength ($p \leq 0.05$) significant increase in the inactivation level in terms of log reduction was seen with increasing treatment time. For instance, at 9 kV/cm field strength, the log reduction increases from 0.27 to 1.32 D

w.r.t. treatment time 100–500 μ s, respectively (Fig. 1). Similarly, in the case of a fixed treatment time 500 μ s, the inactivation level has reached to higher extent from 1.32 to 3.19 D as the field strength is increased from 9 to 21 kV/cm. Log reduction of less than 1 D has been observed at 9–15 kV/cm for 100 μ s. However, PEF treatment at 21 kV/cm for 500 μ s has caused the inactivation of 3.19 D . While the inactivation level increases significantly ($p \leq 0.05$) with increase in field strength at a fixed duration of treatment time (Fig. 1). Thus, it can be inferred that treatment at higher field strength for prolonged period will further enhance the inactivation level.

The survival curves observed at various field strengths were fitted to the corresponding models (Eqs. 1, 3, 5 and 7) to evaluate their kinetic parameters. Determining criticality-process parameters and quality attributes for each model are shown in Tables 1 and 2. As it is clear from the Table 2 that the performance of Bigelow model was good for 100 μ s and it deteriorated afterward. The coefficient of determination at 500 μ s is 0.2609 which indicates that the observed results are far away from the predicted ones. Bigelow model is based on the first order kinetics so it fits well for few $\log_{10}$ cycles reduction. As the $\log_{10}$ increases, the survival curves follow the non-linear pattern and first-order kinetic model fails to describe it.

The coefficients of determination for Peleg and Hülshager models show that these models fit the observed data better than the Bigelow model. The coefficients of determination for Hülshager and Peleg models are comparable, however, on an average; R^2 is greater for Hülshager model

Table 1 Evaluation of kinetic parameters for Peleg and Hülshager models

Treatment time	Peleg model			Hülshager model		
	k	E_c	R^2	b_E	E_c	R^2
100	3.345 ± 0.0847	8.993 ± 0.5353	0.9935–0.9971	0.2410 ± 0.0083	6.5712 ± 0.1983	0.9986–0.9999
200	2.833 ± 0.0053	8.042 ± 0.4105	0.9929–0.9959	0.3086 ± 0.0046	6.4504 ± 0.2681	0.9920–0.9996
300	2.765 ± 0.0647	5.714 ± 0.5072	0.9915–0.9945	0.3408 ± 0.0066	4.833 ± 0.4693	0.9909–0.9954
400	2.741 ± 0.1911	3.911 ± 1.3139	0.9766–0.9832	0.3539 ± 0.0226	3.5266 ± 1.0892	0.9758–0.9829
500	2.543 ± 0.0274	1.663 ± 0.1268	0.9683–0.9739	0.3892 ± 0.0046	1.4743 ± 0.1221	0.9683–0.9739

Table 2 Evaluation of kinetic parameters Bigelow and Weibull models

Field strength (kV cm ⁻¹)	Bigelow model		Weibull model		
	D	R^2	n	a	R^2
9	389 ± 51.55	0.9214–0.9931	1.2979 ± 0.1683	207.57 ± 42.00	0.5284–0.7483
12	287 ± 28.59	0.8170–0.9904	0.8552 ± 0.1901	102.26 ± 17.87	0.4963–0.6957
15	205.61 ± 6.51	0.8377–0.9687	0.7687 ± 0.0552	58.09 ± 6.53	0.8850–0.9086
18	166.45 ± 13.54	0.7162–0.9606	0.6967 ± 0.1419	36.23 ± 21.29	0.6629–0.8616
21	141.55 ± 3.08	0.2350–0.7820	0.5289 ± 0.0889	12.77 ± 7.23	0.8750–0.8839

Fig. 2 Variation of E_c , b_E parameters of Hülshager model with treatment time

Fig. 3 Variation of 'n' and 'a' parameters of Weibull model with field strength

as compared to Peleg model. The critical field intensities which are evaluated for Hülshager and Peleg models, ranged from 6.5712–1.4743 to 8.993–1.663 kV/cm respectively. The evaluated critical field intensity is different from the other reported studies (Hülshager et al. 1981; Martín et al. 1997; Zhong et al. 2005). The cause of disagreement can be the environmental conditions and treatment setup used in these studies.

The observed data when fitted to Weibull model, an accuracy factor of order 1.0851 was observed which indicates a good prediction agreement. The accuracy factor for Weibull model is ~ 1.0 with least deviation among all the described models.

Based on the accuracy factor, Hülshager model and Weibull distribution model are competent to predict the unknown data. In order to predict microbial reduction, at any level of field strength and treatment time, secondary models are developed using Hülshager and Weibull equations. It is clear from Tables 1 and 2 that E_c and b_E parameters of Hülshager model and a and n parameters of Weibull model showed variations with treatment time and electric field strength, respectively. The variations in E_c and b_E parameters of Hülshager model with electric field strength is shown in Fig. 2. An empirical relation of E_c as an exponential function of

treatment time and b_E as logarithmic function of treatment time are found as

$$E_c = 11.846e^{-0.004T} \quad (14)$$

$$b_E = 0.0874 \ln(T) - 0.159 \quad (15)$$

with coefficient of determination 0.8790 and 0.9865 for E_c and b_E respectively. Using Eqs. 14 and 15 in Eq. 5, secondary model for model 3 can be represented as

$$\ln S = -(0.0874 \ln(T) - 0.159)(E - 11.846e^{-0.004T}). \quad (16)$$

The variations of a and n parameters of Weibull model are presented in Fig. 3. These parameters of Weibull model are related to field strength as follows:

$$n = 10.144E^{-0.956} \quad (17)$$

$$a = 1539.7e^{-0.22E} \quad (18)$$

here, E is applied field strength. The coefficient of determination for 'n' and 'a' were 0.9520 and 0.9821. Using Eqs. 17 and 18 in Eq. 7, the secondary model for model 4 can be represented as

$$\ln S = \left(\frac{T}{1539.7e^{-0.22E}} \right)^{10.144E^{-0.956}}. \quad (19)$$

Fig. 4 Validation of secondary models on the observed data

Models given in Eqs. 16 and 19 are validated on the set of observations which are not used for the evaluation of their kinetic parameters. The values of parameters used for prediction were calculated with the use of relationships given in Eqs. 14, 15, 17 and 18. The predicted and observed data are shown in Fig. 4. The accuracy factor for validated data is observed as 1.0585 and 1.0853 for Hülshager and Weibull models respectively. The bias factor of 0.9213 for Weibull and 1.0174 for Hülshager indicates that Weibull model under fit the validation data while the Hülshager model over fits the same. Also, the performance of Hülshager model is better with respect to Weibull model.

The observed data is fitted on four models described in Eqs. 1, 3, 5 and 7. The parameters such as, the accuracy, bias and R^2 values are determined for all the models. The comparison of accuracy factor reveals that Bigelow model is least accurate in predicting the data with accuracy factor of 1.2157 which signifies 21% error in microbial inactivation prediction. At the same time, Peleg and Hülshager models have accuracy factor of 1.1998 and 1.1251 respectively. So it is clear from the accuracy factor, the deviation of predicted results from the observed ones are more for Peleg model as compared to Hülshager model. Nevertheless, both the Peleg and Hülshager models are felicitous for prediction of survival fraction as coefficients of determination are very close to each other and in the range of 0.9713–0.9956 and 0.9713–0.9991, respectively. It is clear from Table 3 that the accuracy factor for Weibull distribution model is 1.0874 with least error of 8.7% among all the described models.

The models used in this study are based on the empirical and phenomenological based consideration. For example, models 1, 2, and 4 are based on the empirical consideration while the model 3 is based on the phenomenological consideration. A major difficulty in the field of predictive microbiology is that the prediction models are not based on the mechanism involved during inactivation process (Baranyi et al. 1996). The process of sterilization with PEF has

Table 3 Calculated performance indices of Bigelow, Peleg, Hülshager and Weibull models

Model	Bias factor	Accuracy factor	MSE
Bigelow	1.125402872	1.215718947	0.0536–0.0645
Peleg	1.087443347	1.199881432	0.0054–0.0065
Hülshager	0.999735635	1.125167147	0.0049–0.0062
Weibull	1.028319041	1.085123728	0.0059–0.0062

been demonstrated well but the underlying mechanism that causes bactericidal effect has not been fully understood yet. In such cases, it is very challenging to develop purely mechanistic predictive models. In terms of R^2 , it can be inferred that the empirical models 3 and 4 fits well with the observed data. However, the goodness of fit is not the only criteria for choosing a predictive model. A predictive mathematical model is optimum, if it is intelligible with few parameters and accommodates the effect of environmental conditions as well.

Model 1 was proposed for thermal inactivation and is based on the first order kinetics. As per this model, the inactivation should follow a linear variation with the casual factor. This model describes the survival curves least accurately among all four models, which is due to the nonlinear path.

Model 2 and 3 described the survival curves appropriately. In these models 2 and 3 observed E_c are 1.66 and 1.58 kV/cm, respectively at 500 μ s. It is noticed from Table 3 that accuracy factor for Hülshager model is better than Peleg model.

Secondary models are developed by relating one or more parameter of primary models with the influencing factor (vide infra) for Hülshager and Weibull models to describe the survival curves of *E. coli* at given field strength and treatment time. In Hülshager model, E_c and b_E are related to treatment time logarithmically and exponentially. Similarly for Weibull model n and a are expressed as power and exponential function of field strength.

Conclusion

This study reveals that, the Hülshager and Weibull models were superior in performance than the Peleg and Bigelow models for predicting the inactivation of *E. coli* in carrot juice. The performance of Hülshager and Weibull models was further improved by developing the secondary models. The formulated secondary models can be used to predict the inactivation of *E. coli* present in carrot juice at different levels using pulse electric field. Although secondary models focused on specific relevant foods or food groups

would produce more realistic models. The environmental factors and mechanism behind the inactivation may be incorporated in these models which may lead to development of a superior model for describing the microbial inactivation or death kinetics of foodborne pathogens more accurately. Lethal rates are required to calculate equivalence between different PEF treatments in terms of inactivation of *E. coli* and to assess if products meet default criteria for pasteurized foods or refrigerated foods of extended durability.

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References

- Abram F, Smelt JPPM, Bos R, Wouters PC (2003) Modelling and optimization of inactivation of *Lactobacillus plantarum* by pulsed electric field treatment. *J Appl Microbiol* 94:571–579. doi:[10.1046/j.1365-2672.2003.01869.x](https://doi.org/10.1046/j.1365-2672.2003.01869.x)
- Akin E, Evrendilek GA (2009) Effect of pulsed electric fields on physical, chemical, and microbiological properties of formulated carrot juice. *Food Sci Technol Int* 15:275–282. doi:[10.1177/1082013209341414](https://doi.org/10.1177/1082013209341414)
- Álvarez I, Virto R, Raso J, Condón S (2003) Comparing predicting models for the *Escherichia coli* inactivation by pulsed electric fields. *Innov Food Sci Emerg Technol* 4:195–202. doi:[10.1016/S1466-8564\(03\)00004-3](https://doi.org/10.1016/S1466-8564(03)00004-3)
- Aronsson K, Rönner U, Borch E (2005) Inactivation of *Escherichia coli*, *Listeria innocua* and *Saccharomyces cerevisiae* in relation to membrane permeabilization and subsequent leakage of intracellular compounds due to pulsed electric field processing. *Int J Food Microbiol* 99:19–32. doi:[10.1016/j.ijfoodmicro.2004.07.012](https://doi.org/10.1016/j.ijfoodmicro.2004.07.012)
- Baranyi J, Ross T, McMeekin TA, Roberts TA (1996) Effects of parameterization on the performance of empirical models used in ‘predictive microbiology’. *Food Microbiol* 13:83–91. doi:[10.1006/fmic.1996.0011](https://doi.org/10.1006/fmic.1996.0011)
- Bigelow WD (1921) The logarithmic nature of thermal death time curves. *J Infect Dis* 25:528–536
- Chen BH, Peng HY, Chen HE (1995) Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. *J Agric Food Chem* 43:1912–1918. doi:[10.1021/jf00055a029](https://doi.org/10.1021/jf00055a029)
- Hülsheger H, Potel J, Niemann E-G (1981) Killing of bacteria with electric pulses of high field strength. *Radiat Environ Biophys* 20:53–65. doi:[10.1007/BF01323926](https://doi.org/10.1007/BF01323926)
- Jayaram S, Castle GS, Margaritis A (1992) Kinetics of sterilization of *Lactobacillus brevis* cells by the application of high voltage pulses. *Biotechnol Bioeng* 40:1412–1420. doi:[10.1002/bit.260401116](https://doi.org/10.1002/bit.260401116)
- Lado BH, Yousef AE (2002) Alternative food-preservation technologies: efficacy and mechanisms. *Microbes Infect* 4:433–440. doi:[10.1016/S1286-4579\(02\)01557-5](https://doi.org/10.1016/S1286-4579(02)01557-5)
- Martín O, Qin BL, Chang FJ et al (1997) Inactivation of *Escherichia coli* in skim milk by high intensity pulsed electric fields. *J Food Process Eng* 20:317–336. doi:[10.1111/j.1745-4530.1997.tb00425.x](https://doi.org/10.1111/j.1745-4530.1997.tb00425.x)
- Peleg M (1995) A model of microbial survival after exposure to pulsed electric fields. *J Sci Food Agric* 67:93–99. doi:[10.1002/jsfa.2740670115](https://doi.org/10.1002/jsfa.2740670115)
- Peleg M, Cole MB (1998) Reinterpretation of microbial survival curves. *Crit Rev Food Sci Nutr* 38:353–380. doi:[10.1080/10408699891274246](https://doi.org/10.1080/10408699891274246)
- Peleg M, Pechina CM (2000) Modeling microbial survival during exposure to a lethal agent with varying intensity. *Crit Rev Food Sci Nutr* 40:159–172. doi:[10.1080/10408690091189301](https://doi.org/10.1080/10408690091189301)
- Rivas A, Sampedro F, Rodrigo D et al (2006) Nature of the inactivation of *Escherichia coli* suspended in an orange juice and milk beverage. *Eur Food Res Technol* 223:541–545. doi:[10.1007/s00217-005-0234-4](https://doi.org/10.1007/s00217-005-0234-4)
- Ross T (1996) Indices for performance evaluation of predictive models in food microbiology. *J Appl Bacteriol* 81:501–508. doi:[10.1111/j.1365-2672.1996.tb03539.x](https://doi.org/10.1111/j.1365-2672.1996.tb03539.x)
- Saldana G, Puertolas E, Monfort S, Raso J, Alvarez I (2011) Defining treatment conditions for pulsed electric field pasteurization of apple juice. *Int J Food Microbiol* 151(1):29–35. doi:[10.1016/j.ijfoodmicro.2011.07.033](https://doi.org/10.1016/j.ijfoodmicro.2011.07.033)
- Toepfl S, Heinz V, Knorr D (2007) High intensity pulsed electric fields applied for food preservation. *Chem Eng Process Process Intensif* 46:537–546. doi:[10.1016/j.ccep.2006.07.011](https://doi.org/10.1016/j.ccep.2006.07.011)
- Van Boekel M (2002) On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *Int J Food Microbiol* 74:139–159. doi:[10.1016/S0168-1605\(01\)00742-5](https://doi.org/10.1016/S0168-1605(01)00742-5)
- Xiang B, Sundararajan S, Mis Solval K et al (2014) Effects of pulsed electric fields on physicochemical properties and microbial inactivation of carrot juice. *J Food Process Preserv* 38:1556–1564. doi:[10.1111/jfpp.12115](https://doi.org/10.1111/jfpp.12115)
- Zhong K, Chen F, Wu J et al (2005) Kinetics of inactivation of *escherichia coli* in carrot juice by pulsed electric field. *J Food Process Eng* 28:595–609. doi:[10.1111/j.1745-4530.2005.00041.x](https://doi.org/10.1111/j.1745-4530.2005.00041.x)