MODELING MICROBIAL INACTIVATION SUBJECTED TO NONISOTHERMAL AND NON-THERMAL FOOD PROCESSING TECHNOLOGIES

by

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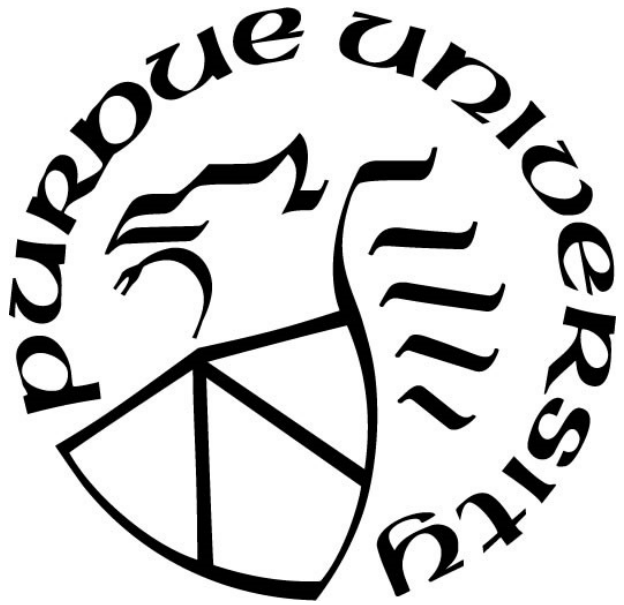

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Dedicated to my beloved ones. To my parents, Angela and Rafael, who instilled in me the values of hard work, resilience and perseverance, which contributed immensely to my pursuit of knowledge and education. And to my brothers, Lucas and Pedro, who have always been excited and proud of my achievements.

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ABSTRACT

Author: Mendes Candido de Oliveira, Gabriella. PhD
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Title: Modeling Microbial Inactivation Subjected to Nonisothermal and Non-thermal Food Processing Technologies
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Modeling microbial inactivation has a great influence on the optimization, control and design of food processes. In the area of food safety, modeling is a valuable tool for characterizing survival curves and for supporting food safety decisions. The modeling of microbial behavior is based on the premise that the response of the microbial population to the environment factors is reproducible. And that from the past, it is possible to predict how these microorganisms would respond in other similar environments. Thus, the use of mathematical models has become an attractive and relevant tool in the food industry.

This research provides tools to relate the inactivation of microorganisms of public health importance with processing conditions used in nonisothermal and non-thermal food processing technologies. Current models employ simple approaches that do not capture the realistic behavior of microbial inactivation. This oversight brings a number of fundamental and practical issues, such as excessive or insufficient processing, which can result in quality problems (when foods are over-processed) or safety problems (when foods are under-processed). Given these issues, there is an urgent need to develop reliable models that accurately describe the inactivation of dangerous microbial cells under more realistic processing conditions and that take into account the variability on microbial population, for instance their resistance to lethal agents. To address this urgency, this dissertation focused on mathematical models, combined mathematical tools with microbiological science to develop models that, by resembling realistic and practical processing conditions, can provide a better estimation of the efficacy of food processes. The objective of the approach is to relate the processing conditions to microbial inactivation. The development of the modeling approach went through all the phases of a modeling cycle from planning, data collection, formulation of the model approach according to the data analysis, and validation of the model under different conditions than those that the approach was developed.

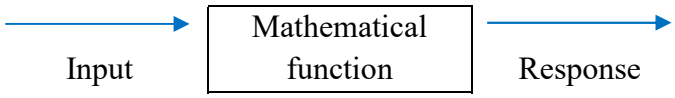
A non-linear ordinary differential equation was used to describe the inactivation curves with the hypothesis that the momentary inactivation rate is not constant and depends on the instantaneous processing conditions. The inactivation rate was related to key process parameters to describe the inactivation kinetics under more realistic processing conditions. From the solution of the non-linear ordinary differential equation and the optimization algorithm, safety inferences in the microbial response can be retrieved, such as the critical lethal variable that increases microbial inactivation. For example, for nonisothermal processes such as microwave heating, time-temperature profiles were modeled and incorporated into the inactivation rate equation. The critical temperature required to increase the microbial inactivation was obtained from the optimization analysis. For non-thermal processes, such as cold plasma, the time-varying concentration of reactive gas species was incorporated into the inactivation rate equation. The approach allowed the estimation of the critical gas concentration above which microbial inactivation becomes effective. For Pulsed Electric Fields (PEF), the energy density is the integral parameter that groups the wide range of parameters of the PEF process, such as the electric field strength, the treatment time and the electrical conductivity of the sample. The literature has shown that all of these parameters impact microbial inactivation. It has been hyphothesized that the inactivation rate is a function of the energy density and that above a threshold value significant microbial inactivation begins.

The differential equation was solved numerically using the Runge-Kutta method (*ode45* in MATLAB ®). The *lsqcurvefit* function in MATLAB ® estimated the kinetic parameters. The approach to model microbial inactivation, whether when samples were subjected to nonisothermal or to non-thermal food processes, was validated using data published in the literature and/or in other samples and treatment conditions. The modeling approaches developed by this dissertation are expected to assist the food industry in the development and validation process to achieve the level of microbial reduction required by regulatory agencies. In addition, it is expected to assist the food industry in managing food safety systems through support food safety decision-making, such as the designation of the minimal critical parameter that may increase microbial inactivation. Finally, this dissertation will contribute in depth to the field of food safety and engineering, with the ultimate outcome of having a broad and highly positive impact on human health by ensuring the consumption of safe food products.

CHAPTER 1. INTRODUCTION

1.1 What is model, why models, and how are models built: the modeling cycle

A mathematical model is a mathematical expression that builds a relationship between dependent and independent variables. This relationship involves constants (or parameters) that may or may not be related to intrinsic and/or extrinsic factors (Brandão & Silva, 2009). The goal of a mathematical model is to describe real systems (a physical model) by using mathematical equations based on the most significant physical characteristics of the systems. As a result, responses of the systems to external input conditions can be estimated by the mathematical equations describing and representing the physical models. For a given system or process, a mathematical model estimates its response based on the values of the input variables (Pérez-Rodríguez & Valero, 2013) according the following schematic structure:



A model may also be a simplified description of a relationship between observations of the system (responses) and the input factors that are more likely to cause the observed responses. In addition to describing a collection of data, a mathematical model may also represent a hypothesis or series of hypotheses about underlying relationships between the independent variables that lead to the response or observations (data). The modeling approach describing the data without a physical representation of the system is termed as 'empirical' model (McMeekin et al., 2008). Empirical models, therefore, simply describe the data with a generic mathematical relationship, often as a polynomial expression (Ross, McMeekin, & Baranyi, 2014). Whereas the approach of summarizing the understanding or knowledge that leads to the observations is called 'mechanistic' (or 'deterministic') model (McMeekin et al., 2008). In other words, 'mechanistic' models are based on fundamental mechanisms involved in a process defining the system under study (Brandão & Silva, 2009). Both approaches are capable of predicting the response of the system to the changes in the variables and have been widely applied in food engineering (McMeekin et al., 2008).

The development of sound modeling approaches usually goes through a modeling cycle basically performed in four phases: (1) planning, (2) data collection, (3) mathematical description,

and (4) model validation. The first phase, planning, involves gathering of existing knowledge that can explain and/or simplify observations of the system behavior, e. g. microbial behavior (Valdramidis, 2016). The next phase is data collection. If the treatment conditions or experimental design to collect the data are done wisely, the adequacy of the model can be tested, the parameter estimation can be improved and a clear assessment of the main variables affecting the system can be obtained. However, if the experimental data set is poor, no sophisticated modeling or complex data analysis will provide a realistic modeling approach (Brandão & Silva, 2009). Following experimental data collection, data analysis is the stage used to formulate the mathematical model. The suitability of the model is assessed by defining a function that is a suitable measure of the deviation between the experimental data and the values calculated by the model. At this phase, the objective is to minimize that deviation in order to calculate/optimize the model parameters that achieve that minimum. Thus, this optimization is the core value of parameter estimation. The least-square method is the common routine used for optimization which consists of finding the parameter values that minimize the squared difference between the model predicted values and the experimental data (Brandão & Silva, 2009). A suitable model needs to be in line with the accuracy of the fit, has to be able to predict the untested combination of factors, incorporate all relevant factors, possesses a minimum number of parameters, a low correlation between the parameters as well as providing parameters that are realistic and with biological meaning. Reparameterization for improving statistical properties should also be considered during model development (Valdramidis, 2016). The final phase of a modeling cycle is model validation. Model validation involves comparing model predictions with analogous observations, but different than the ones used to develop the model (Ross et al., 2014). In other words, a mathematical model is validated when is applied to successfully describe more complex systems, running at experimental conditions different from those used to develop the model (Valdramidis, 2016). Data for model validation can be generated using different conditions or may come from the literature (Guillier, 2016). Models can also be validated in media, but especially, they should be validated in real food products (Devlieghere, Francois, Vermeulen, & Debevere, 2009). In this way, model predictions can be used to predict the results at a set of conditions, to test the hypotheses incorporated in the model and, if necessary, to revise and improve the model and the hypotheses if the predictions do not match the observations (data). Finally, modeling provides a systematic way to improve the understanding of the underlying processes, and with that

understanding, the researcher is better prepared to predict, control, or improve system performance wisely (McMeekin et al., 2008).

1.2 The role of modeling in food safety

Food safety is a complex area that depends on several factors. Interconnected factors like environmental, cultural or socioeconomic factors can lead to exposure to dangerous microorganisms. Factors related to the human host (host's age, gender, health, and dietary practices), or related to the handling of the food during preparation in the home or in the food service establishment, or related to food handler hygiene, or to water quality, can also result in unsafe foods. Harmful microbes or pathogens can be also introduced during processing and handling or through other cross-contamination routes such as between fresh and cooked foods. Hazardous levels can increase during distribution and storage if these steps are not properly done. Food safety is, therefore, not an issue only for microbiologists. Nowadays, the scientific community is moving towards gathering multidisciplinary teams with a wide range of expertise, from microbiology, engineering, genetics, and immunology, to understand the interrelated factors that lead to the emergence of food safety issues (IFT EP, 2000).

Foodborne disease is a major cause of morbidity worldwide, resulting in significant costs in both developed and developing countries (Käferstein & Absussalam, 1999). The United States' approach to managing food safety has contributed to its classification as one of the safest in the world (IFT EP, 2000). However, the incidence of foodborne diseases is an issue not fully solved. Numerous outbreaks have been harming the American population up to our days. Only in 2019, for instance, the Center for Diseases Control and Prevention (CDC) reported outbreaks in a variety of products: in raw milk outbreak due to *Brucella* RB51 was reported with one case confirmed in New York and potentially hundreds of people were exposed to RB51 in 19 states, *Salmonella* infections were linked to pre-cut melons in 10 states and 137 people infected, ground beef products were linked to *Escherichia coli* O103 in 10 states and 196 people infected, imported oysters contaminated with multiple pathogens (*Vibrio*, *Shigella*, *norovirus*, STEC, and *Campylobacter*) infected 16 people in 5 states, *Salmonella* Concord infections were linked to imported Karawan brand in products made with tahini such as hummus, *E. coli* O26 infections were linked to flour in 8 states and 17 people infected (CDC, 2019). Clearly, the existing approach to food safety management is, in some cases, insufficient to prevent food-related outbreaks.

CDC estimates the burden of foodborne diseases with alarming numbers of 48 million people getting sick, 128 thousand being hospitalized and 3 thousand dying from foodborne illnesses each year in the United States (CDC, 2018). Furthermore, unsafe foods represent a global health threat. According to the World Health Organization (WHO) (2019), 550 million people get sick and 230 thousand deaths are due to the consumption of contaminated foods. The most vulnerable people are infants, young children, pregnant women, elderly, and those with an underlying illness. Within the 220 million children that contract diarrheal diseases, 96 thousand die every year (WHO, 2019). The complex nature of food safety ensures that new challenges will continue to emerge (IFT EP, 2000).

In the area of food safety, modeling has become increasingly important (Brandão & Silva, 2009). The modeling of microbial behavior (growth or inactivation) is based on the premise that the response of the microbial population to the environment factors is reproducible. And that from the past, it is possible to predict how these microorganisms would respond in other similar environments (Ross et al., 2014).

Mathematical models compromise a wide range of applications, such as the description of the introduction of the pathogens in foods, the microbial growth over time, the inactivation of microorganisms exposed to traditional or innovative treatments, or the use of models to describe the consumption of microorganisms that the food is carrying and the subsequent illness (Gougouli & Koutsoumanis, 2016).

The development of mathematical models and their application in food safety fits into the discipline of predictive microbiology, or predictive modeling in foods (Membré & Valdramidis, 2016). Predictive microbiology, in turn, combines disciplines of food microbiology, engineering, and statistics, which combine tools to provide predictions of the behavior of microorganisms in food systems (Schaffner & Labuza, 1997).

The foundation for managing food safety and quality systems is composed of prerequisite programs (e.g. good manufacture practices and good hygiene practices), and the Hazard Analysis Critical Control Points (HACCP) plan (Membré & Valdramidis, 2016). Quantitative microbiological tools for food safety management have become an attractive and pertinent tool in the food industry (Gougouli, & Koutsoumanis, 2016). Thus, mathematical models have a vast range of potential applications within the food industry. Current applications are summarized in Table 1 (Pérez-Rodríguez & Valero, 2013).

Table 1. Potential application of mathematical models

Application	Context
Hazard Analysis and Critical Control Points (HACCP)	- Preliminary hazard analysis - Identification and establishment of critical control points - Corrective measures - Evaluation of variables interaction
Risk Assessment and Risk Management	- Estimation of microbial population dynamics along the food chain - Exposure assessment toward a specific pathogen - Design of scientifically based management strategies to assure food safety
Shelf life studies	- Growth prediction of spoilage or pathogenic microorganisms in foods
Innovation and development of a new product	- Evaluation of the impact of microbial spoilage in a food product - Effect of processing on food quality and safety - Evaluation of the effect of other additional factors throughout the food chain
Hygienic measures and temperature integration	- Evaluation of the consequences of chill chain application on microbial spoilage - Optimization of thermal and nonthermal inactivation processes
Education	- Education of both scientific and nonscientific staff - Implementation and training of computing-based decision systems
Experimental design	- Estimation of the number of samples to be prepared - Definition of intervals within each factor to be analyzed

In an industrial perspective, Membré and Lambert (2008) categorized the applications of predictive modeling into three groups: (1) Product innovation and development, (2) Operational support, and (3) Incident support.

1. Predictive models can be used to develop new products and processes, to formulate existing products, to determine storage conditions and shelf-life, by the assessment of the growth of spoilage and pathogenic microorganisms, assessing growth limits, or inactivation rates associated with particular food formulations and/or process conditions;
2. Predictive models support food safety decisions when implementing or executing a food manufacturing operation. Decisions such as the design of in-factory heating regimes and the setting of critical control points (CCPs) in HACCP are just a few examples. In addition, predictive models support the assessment of the impact of process deviations on microbiological safety and the quality of food products;
3. Predictive models can estimate the impact on consumer safety or product quality in case of problems of products occurring in the market. Thus, models are useful in incident reporting.

The importance of mathematical models for the food industry (HACCP, shelf-life determination, product formulation) and their risk assessment capability has been explicitly endorsed by regulatory agencies, e.g. European Commission Regulation (EC) No2073/2005 referred to *the use of predictive mathematical modeling established for the food in question, using critical growth or survival factors for the micro-organisms of concern in the product* (Membré & Valdramidis, 2016).

As predictive models provide an alternative to the traditional microbiological assessment of food safety and quality and product development continues to grow in most food manufacturing areas, the use of mathematical models has become a useful tool recognized by leading food industries. Unilever and Nestlé, for instance, have used predictive microbiology in a variety of applications (Membré & Lambert, 2008). To name a few, Unilever developed a model of probabilistic exposure assessment to assess the safety of refrigerated processed foods of extended durability in relation to *Bacillus cereus*. The model was structured to provide the probability of having 5 log CFU/g or more of *B. cereus* at the point of consumption (Membré, Kan-King-Yu, & Blackburn, 2008). Nestlé developed a modeling software to predict growth/no-

growth of *Salmonella* Poona as a function of pH, salt, acetate, and sorbate. The model calculated the minimum pH (3.81) and minimum a_W (0.948) for the growth of *Salmonellae* agreed with the range of values reported in the literature (Lambert & Bidlas, 2006; Membré & Lambert, 2008).

Within a safety frame, mathematical modeling is a continuously developing tool in the food industry and is far from being complete. The valuable role of mathematical modeling to assist design, control, and optimization of food processes, as well as supporting the management of food safety systems will continue, as well as other scientific developments in modeling, microbiology, and food engineering techniques (Membré & Lambert, 2008). With the well-guided and interdisciplinary work between scientists, microbiologists, engineers, and statisticians, the use of modeling approaches to a wide range of process endeavors will be achievable (Brandão & Silva, 2009).

1.3 Research motivation, objectives and expected impact

Models describing the inactivation of pathogenic microorganisms are indispensable for designing processes to produce safe foods. Conventionally, the efficacy of food processes is based on the assumption that survival curves can be represented by a linear equation. From this equation, a theoretical value is assumed to predict the time needed to reduce the microbial population by a factor of ten. However, the calculation of this value has several flaws that can lead to undesirable consequences in the food product, including increasing the risk of microbial contamination if the product is underprocessed, or reducing the amount of nutritious compounds if the product is overprocessed. The deficiency of linear or first-order kinetic models also underlies in the theories on which they are based, such as that the microbial population is completely identical, and that a uniform lethal agent would inactivate all the organisms at the same time. Unfortunately, ample evidence indicates that the microbial population consists of several subpopulations, each with its own spectrum of resistance. These different patterns result in survival curves with different shapes, which leads to the emergence of non-linear survival curves. For this reason, there is a *gap* in the conventional approach since it cannot accurately describe the inactivation of microbial population in realistic scenarios. Therefore, there is an *urgent need* to develop reliable mathematical models that describe real systems by using mathematical equations based on the most important properties of the system. To address

this urgency, the dissertation developed a modeling approach that relates the process variables to the description of the microbial inactivation. This work focused on nonisothermal and non-thermal food processes because none of the modeling approaches reported in the literature provides a useful application.

The design of effective food treatments that promote the safety level required by U.S. FDA regulations depends on the knowledge of the kinetics of microbial destruction. Thus, the *overall goal* of this research was to develop, evaluate and validate a mathematical modeling approach that accurately describes the inactivation kinetics of microorganisms subjected to traditional and innovative technologies. The *objective of this dissertation* was to develop a flexible modeling approach capable of incorporating the process variables into the description of microbial inactivation in nonisothermal and non-thermal food processes. The *specific aims* were to (1) model the inactivation of spoilage bacterial spores such as *Bacillus subtilis* subjected to cold plasma sterilization; (2) to model the inactivation of microorganisms of public health concern, such as *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in apple juice processed with microwave heating, and (3) to model the inactivation of these pathogenic cells under Pulsed Electric Fields (PEF).

This research has a key impact on the food industry as it is aimed to develop the best set of process conditions to reduce the incidence of costly food-related outbreaks. The modeling approaches developed in this dissertation are expected to assist the food industry in the development and validation process to achieve the level of microbial reduction required by regulatory agencies. In addition, it is expected to assist the food industry in the management of food safety systems by supporting food safety decisions, such as defining critical parameters that can enhance microbial inactivation when the product is subjected to the technologies investigated by this research. In addition to assist the establishment of critical control points in HACCP systems for microwave, PEF, and cold plasma, and to indicate which process factors need to be monitored during the production process. Finally, this dissertation will contribute in depth to the field of food safety and engineering, with the ultimate outcome of having a broad and highly positive impact on human health by assuring the consumption of safe food products.

1.4 Organization of the Dissertation

The content of this dissertation is organized in six chapters which are briefly summarized as follows:

Chapter 1 provides background on mathematical models, their role in food safety, and their vast potential applications in the food industry. Research motivation, objectives, and expected impacts are introduced.

Chapter 2 presents a review of mathematical models for microbial inactivation applied in traditional and innovative food processes. Critical review of current models used for microbial inactivation in microwave heating, pulsed electric fields, and cold plasma processes are discussed along with the identified gaps in the literature.

Chapter 3 proposes a modeling approach to describe the inactivation curves of *Bacillus subtilis* spores under cold plasma sterilization, correlating the inactivation kinetics with the dynamic conditions of one of the important lethal gas specie originated in the cold plasma process. The modeling approach is validated with published data obtained from the literature.

Chapter 4 proposes a modeling approach to describe the inactivation curves of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium under microwave pasteurization of apple juice at temperatures between 80 to 90°C. The modeling approach was able to describe the inactivation kinetics with the nonisothermal characteristics of microwave treatments. The validation of the modeling approach was performed in other juice samples inoculated with the target microorganisms and processed under different conditions.

Chapter 5 proposes a modeling approach to describe the inactivation curves during Pulsed Electric Fields. Apple cider inoculated with *Escherichia coli* O157:H7 or *Salmonella* Typhimurium was used to build the modeling approach. The approach correlated the PEF process parameters with the inactivation characteristics. The proposed modeling approach was validated in other samples, and in other juices with data obtained from the literature.

Chapter 6 discusses the general conclusions of this work, in addition to give recommendations for future research.

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CHAPTER 2. LITERATURE REVIEW

Food process engineering aims not only to create new products but also to improve the quality of the existing ones to satisfy consumer demands (Rahman & Ahmed, 2012). Thermal processing is the most common method used as a preservation method in food processing to obtain convenient and safe products with minimal risk of having harmful bacteria (Rosnes, Fernandez, Periago, & Skara, 2012). Among the benefits of using thermal processing, the inactivation of foodborne pathogens and the extension of shelf-life boost the application of the thermal process in several types of products. A complete review of the benefits of thermal processing can be viewed in van Boekel et al. (2010). Although conventional thermal processes such as canning and pasteurization effectively inactivate microorganisms and enzymes, negative impacts reported on the color, taste and nutritional quality of foods prevent consumer demands from being met (Misra, Schlüter, & Cullen, 2016). Novel emerging technologies are currently under investigation to substitute conventional pasteurization to fulfill the expanding consumer demands for safe foods with high nutritional value and "fresh-like" taste (Carbonell-Capella, Buniowska, Esteve, & Frígola, 2017; Toepfl, Siemer, Saldaña-Navarro, & Heinz, 2014). The quantification of the microbial inactivation is one of the main areas targeted by food process calculations. Microbial thermal death has been modeled since 1921 starting with the Bigelow model (McKellar & Lu, 2004; Membré & Valdramidis, 2016). Throughout the years, different mathematical models, based on empirical or biological concepts, have been proposed to describe microbial inactivation (Skandamis & Panagou, 2017).

This dissertation focuses on the modeling of inactivation kinetics of emerging technologies that are included in the nonisothermal or non-thermal categories, such as microwave heating, pulsed electric fields, and cold plasma. This chapter gives an overview of the previous and the current modeling approaches used to model microbial inactivation. The subsequent chapters propose a new modeling approach for microwave heating, pulsed electric fields, and cold plasma.

2.1 Classical Linear Models

In 1908, the first-order linear model for bacteria disinfection was proposed by Chick (1908). In thermal processing, it is assumed that thermal destruction of microorganisms follows the kinetics of a first-order chemical reaction, i.e. mathematically modeled with the assumption that the rate of the chemical reaction is proportional to the concentration of one key reactants. Concerning food processing, the first-order kinetics approach was first established to quantify *Clostridium botulinum* spore's inactivation in low acid canned products (McKellar & Lu, 2004) and have been the modeling basis of the canning industry for over 90 years (Ross et al., 2014; Skandamis & Panagou, 2017). Eq. (1) is the mathematical expression to describe a first-order kinetics model:

$$\begin{cases} \dfrac{dN(t)}{dt} = -k'\,N(t) & (0 \le t < +\infty) \\ N(0) = N_0 & (N_0 > 0; t = 0) \end{cases} \tag{1}$$

$N(t)$ and N_0 are the microbial concentration at time t and zero, respectively; k' is the inactivation rate constant. If the inactivation rate constant k' is considered constant over time, integration of Eq. (1) for a fixed time period is expressed as:

$$\int_{N_0}^{N_f} \frac{dN(t)}{N(t)} = \int_{t_0}^{t_f} -k'\,dt \tag{2}$$

The integration of the above equation results in the following equation:

$$N(t) = N_0 \exp(-k't) \tag{3}$$

Another way of expressing Eq. (3) is by defining the survival ratio or microbial reduction $S(t) = N(t)/N_0$ and taking the natural logarithm to yield (McKellar & Lu, 2004):

$$\ln S(t) = \ln\left(\frac{N(t)}{N_0}\right) = -k't \tag{4}$$

Since it is easier to think in microbial destruction quantified by factors of 10 (e.g. 10, 100, 1000, etc), the model is expressed as decimal logarithm:

$$\log S(t) = \frac{-k't}{ln10} = -k\,t \tag{5}$$

Which can be further rearranged into:

$$\log S(t) = -\frac{1}{D}t \tag{6}$$

Where D is the decimal reduction time ($D = 2.303/k'$, units in minutes or seconds). Theoretically, inactivation data and the first-order model are schematically depicted in Fig. 1.

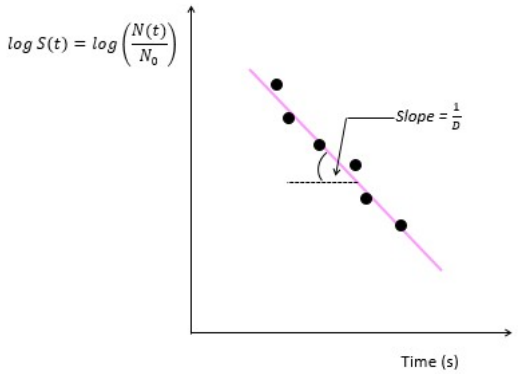

Fig. 1. Linear survival curve. Dots represent the theoretical data, and the line the linear model to determine the D-value.

A linear fit applied to the data is used to determine the value of D either graphically or by running a linear regression routine on the experimental data. The D-value is known as the decimal reduction time, e.g. the time required to decrease the number of microorganisms by a factor of 10 (or to reduce their population by 90%). D-values are specific to the temperature, to the microorganism and to the food product (Morgan, Lund, & Singh, 2010). A drawback of this model is that it is only valid for determining the death of microorganisms at a specific temperature. This means that the product would instantaneously be heated to a specific temperature, held for a fixed time (t minutes or seconds), and then cooled instantaneously. Obviously, a process impossible in practice (Morgan et al., 2010). D-value is also a way to look at the heat resistance of microorganisms. For instance, microorganisms with a higher D-value have a higher heat resistance (Kumar & Sandeep, 2014). D-values can be expressed as (McKellar & Lu, 2004):

$$D = \frac{t}{Log\ N_0 - Log\ N_t} \tag{7}$$

By plotting the log D-values against temperatures, provided the plot is a straight line, the reciprocal of the slope can be calculated, and its value is called the z-value. z is the change in temperature (°C) required to reduce the value D by 90%. In other words, z is the temperature increase needed to reduce D by a factor of 10, so as to increase the destruction rate by a factor of 10. The z value can be calculated following Eq. (8) (Pérez-Rodríguez & Valero, 2013) as:

$$z = \frac{T_{ref} - T}{Log\ D - Log\ D_{ref}} \tag{8}$$

D_{ref} is the D-value at the reference temperature, T_{ref} (121.1°C or 250°F for sterilization). The reference temperature is specified at 121.1°C (250°F) because 90% of the *Clostridium botulinum* spore population is inactivated in 0.21min at that temperature (Morgan et al., 2010).

The effect of temperature on D-value is described by Eq. (9), which rearranged gives the thermal-death-time model (Eq. 9). This model is based on early work by Bigelow (1921).

$$Log\left(\frac{D_T}{D_{ref}}\right) = \frac{T_{ref} - T}{z} \tag{9}$$

$$D_T = D_{ref}\ 10^{\frac{(T_{ref} - T)}{z}} \tag{10}$$

D_T is the D-value at temperature T, and Eq. (10) assumes that z-value is constant for all temperatures.

D and z-values are considered the basis of thermal process calculations and are used to design thermal processes (Kumar & Sandeep, 2014). The ratio of D_{ref} and D_T is the lethal rate or lethality factor L (Kumar & Sandeep, 2014):

$$L = 10^{\frac{T - T_{ref}}{z}} \tag{11}$$

Thermal death time (TDT) or F value is the process time, in a given food at some reference temperature T and z-value, to achieve the desired log reduction in microorganisms (Kumar & Sandeep, 2014). Referring to Eq. (2) and substituting Eq. (10) in place of k', results in (Morgan et al., 2010):

$$\int_{N_0}^{N_f} \frac{-1}{N}\, dN = \int_{t_0}^{t_f} \frac{2.303}{D_{ref}\ 10^{\frac{(T_{ref} - T(t))}{z}}}\, dt \tag{12}$$

F value can be calculated in terms of lethal rate for the process when the temperature T is function of time as (Kumar & Sandeep, 2014):

$$F^{z}{}_{ref} = \int_0^t L\ dt = \int_0^t \left(10^{\frac{T(t) - T_{ref}}{z}}\right) dt = -D_{ref} \log \frac{N_t}{N_0} \tag{13}$$

For a constant temperature process, for instance in an insulated holding tube, T(t) is approximately constant (Morgan et al., 2010), the above equation reduces to (Kumar & Sandeep, 2014):

$$F^{z}_{ref} = t_{hold}\, 10^{\frac{T-T_{ref}}{z}} \quad (14)$$

For the reference temperature 121°C (250°F) and the z-value 10°C (18°F) the F value is referred to as F_0 (Kumar & Sandeep, 2014; Linton, 2010).

Regarding the production of low-acid canned foods (pH > 4.5), the F value is usually set at a 12D value to give a theoretical 12 log cycle reduction of *C. botulinum,* one of the most heat-resistant and perhaps the more lethal spore-forming microorganism in canned food. *C. botulinum* is an anaerobic pathogen that could grow in anaerobic conditions inside a sealed container and then produce the botulinum toxin, which is 65% fatal in humans (Fellows, 2009). Because of this extreme hazard toxin, a 12D process has traditionally been considered a requirement for public health protection (Ahmed, Dolan, & Mishra, 2012). This implies, for example, that if a canned food has 1000 spores and a 12D process is applied, the initial 10^3 spores would be reduced to a theoretical 10^{-9} living spores per can, or in theory, one living spore per 10^9 cans of the product (one spore per one billion cans) (Fellows, 2009). The $D_{121°C}$ for *C. botulinum* is 0.21min, thus the F_0 for a 12D process is 2.52min. Combinations of time and temperature can yield an equivalent lethality. For instance, Eq. (14) can be used to find out what would be the hold time or the F value for a process at 275°F, or at 225°F, to yield a 12D process. The corresponding values would be 0.103min and ~62min, respectively. Usually, a 12D reduction is used for commercial sterility and a 6D reduction is used for pasteurization (Tucker, 2012).

For almost 100 years since Bigelow works in 1921, the first-order kinetic model with D, z and F values have been used to quantify the inactivation of several pathogens in a variety of foods. To name some examples, *Escherichia coli* O157:H7 in ground beef and chicken (Juneja, Snyder, & Marmer, 1997), in apple juice (Splittstoesser, McLellan, & Churey, 1996), strawberry puree (Hsu, Huang, & Wu, 2014), in fish (Rajkowski, 2012); *Salmonella* spp. in chicken (Juneja, Eblen, & Ransom, 2001; Murphy, Marks, Johnson, & Johnson, 2000), peanut butter (Li, Huang, & Cheng, 2014), fish (Rajkowski, 2012); *Listeria monocytogenes* in chicken meats (Murphy et al., 2000), sausages (Felício et al., 2011); *Yersinia enterocolitica* in liquid egg products (Favier, Escudero, & de Guzman, 2008), were modelled by first-order kinetics. However, the calculation of those values has several flaws that can lead to undesirable consequences on the food product.

First, the association between F value, survival ratio, and reference temperature is erroneous when the log survival curve is not log linear. Consequently, for such scenarios, the

D-value has no meaning (Peleg, 2006). Survival curves deviated from first-order kinetics, as having shoulders or tails, upward or downward curvatures, have long been discussed (Cerf, 1977; Moats, Dabbah, & Edwards, 1971; Peleg & Cole, 1998; van Boekel, 2002). Microorganisms that are examples of such deviation are: *Clostridium botulinum* (Anderson, McClure, Baird-Parker, & Cole 1996), *Escherichia coli* (Juneja & Marks, 2005; Kaur, Ledward, Park, & Robson, 1998), *Salmonella* spp. (Buzrul & Alpas, 2007; Humpheson, Adams, Anderson, & Cole, 1998), *Listeria monocytogenes* (Buzrul & Alpas, 2007; Chiruta, Davey, & Thomas, 1997), and *Staphylococcus aureus* (Buzrul & Alpas, 2007). The use of the D-value as a standard method to design thermal process, when the survival curve clearly deviates from linearity is a flaw (Peleg, 2006) that could seriously mislead processing calculation (Moats et al., 1971).

In addition, the approach of ignoring the curvature of the survival curve and forcing a straight line through the data would lead to a number of problems, such as over or under processing of the food (Peleg & Penchina, 2000). For example, as illustrated in Fig. 2(a), in the cases of an upward concavity, forcing a straight line through the data could be a safe approach to ensure the inactivation of the microorganisms given that more than the necessary heating time would be applied. However, the approach can also lead to excessive processing of food and, as a result, the degradation of nutrients and vitamins sensitive to heat. Conversely, when the survival curve follows a downward concavity, Fig. 2(b), forcing a straight line through the data can lead to insufficient processing time to inactivate targeted microorganisms. Although this processing condition would not negatively affect nutrients as much, it would certainly endanger the safety of the product (Peleg, 2006; Skandamis & Panagou, 2017).

Another argument for the inadequacy of the use of first-order kinetics (and D-values) is based on the mechanism underlying the use of this model to describe microbial inactivation. The first-order kinetics assume that the lethal agent acts by destroying a single target in a theoretical homogenous microbial population (Stringer, George, & Peck, 2000). As a result, the cell may have two different fates; either the target is not sufficiently damaged and the cell is alive, or the target is damaged, and the cell is dead. This assumption is not true, since an intermediate level, such as sublethal injury has long been reported, for instance, for *Escherichia coli* O157:H7 (Bromberg, George, & Peck, 1998). The assumption of a homogenous population, in practice, is also a debatable assumption because if all treated cells

are alike or completely identical, then a uniform lethal temperature would kill or inactivate all the organisms at the same time. This pattern of mortality has rarely or never been observed (Peleg, 2006). However, studies have shown the opposite, bacterial cells or spores are inactivated sooner or later than others due to the existence of a spectrum of resistance to the treatment within the microbial population (Peleg & Cole, 1988). If the population of bacteria or spores has a spectrum of resistance, then the inactivation is ultimately a random process (Fredrickson, 1966). The microbial distribution of resistance to the treatment indicates that the microbial population consists of several subpopulations, each with its own inactivation patterns. These different patterns result in survival curves with different shapes, which leads to the rise of non-linear survival curves (Ahmed et al., 2012). This emphasizes the need for models capable of describing the shape of the survival curves and having predictive ability. All that can be concluded at this point is that only when the underlying assumption that the microbial population is homogenous, and, therefore, the organisms are inactivated at the same time, could be verified experimentally for a whole lethal range, the first-order kinetic model with D, z and F values will be valid for the calculation and design of the thermal processes.

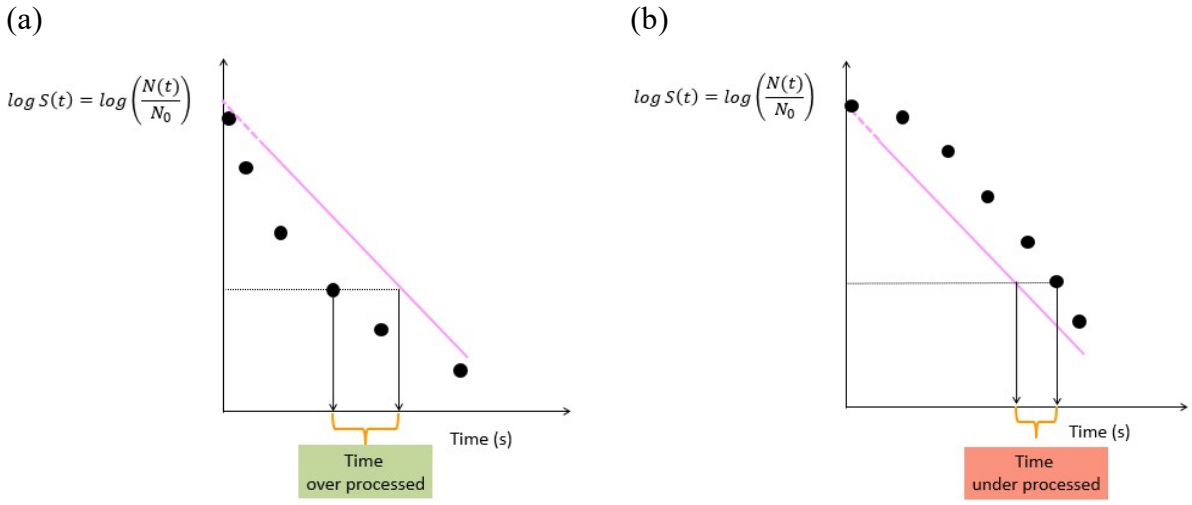

Fig. 2. Schematic representation of inactivation curves with upward (a) and downward (b) concavities. Dots represent the theoretical data and pink line the linear model.

2.2 The Non-linear Case

The deviation of the linearity of the survival curves led to the development of several alternatives to the first-order models. Among them are the modified Gompertz equation (Bhaduri et al., 1991), the biphasic (Cerf, 1977), the Buchanan (Buchanan, Golden, & Whiting, 1993), log-logistic (Cole, Davies, Munro, Holyoak, & Kilsby, 1993) and the Baranyi model (Baranyi & Roberts, 1994). These and other alternative models have been extensively reviewed by Li, Xie, and Edmondoson (2007), and by Xiong, Xie, Edmondson, and Sheard (1999). However, those models did not get much popularity because they can only be used for specific situations, and only for certain microorganisms (Li et al., 2007). The model that gained widespread acceptance, for modeling log-linear and non-linear survival curves for a variety of microorganisms and treatments, due to its simplicity and flexibility, is the Weibull model (Ahmed et al., 2012).

2.2.1 Weibull model

Traditionally, the Weibull distribution is used to describe the time to failure in electronic and mechanical systems. But not only that, its application can be also extended for the analysis of survival data (van Boekel, 2002). In this regard, the inactivation process is seen as a failure phenomenon in which the microorganism fails to resist the adverse conditions after a certain time (Peleg, 2006). In order words, the Weibull model provides a statistical account of a failure time distribution (van Boekel, 2002). This feature makes sense and justify the use of the Weibull distribution to describe survival curves because there probably no single cause of death, that is, many different biophysical processes that cause the death or inactivation of organisms, either directly or through a cascade of events, must exist which characterizes a failure distribution (Peleg, 2006; van Boekel, 2002). The Weibull model can be written by the following equation (Peleg & Cole, 1998):

$$\log S(t) = -\, b\, t^n \tag{15}$$

$S(t)$ is the survival ratio, b the scale parameter, and n the shape parameter. The parameter b can be considered as a non-linear rate constant which reflects the steepness of the isothermal survival curve for the case when n is fixed (Peleg, 2006). The shape of survival curve can be described by n. Upward concave curves have values of $n < 1$, downward concave curves have values of $n > 1$, and when $n = 1$ the survival curve will appear as linear, thus, the appearance of

a first-order model (Peleg & Cole, 1998). Another way of expressing the Weibull for isothermal survival curves of a microbial population is:

$$\log S(t) = -\, b(T)\, t^{n(T)} \tag{16}$$

where $b(T)$ and $n(T)$ can be temperature-dependent constants (Peleg & Penchina, 2000).

The Weibull model has been used to model the inactivation of *Bacillus cereus* spores, *Bacillus stearothermophilus* spores, *Campylobacter jejuni, Clostridium botulinum* spores, *Clostridium sporogenes* spores, *Escherichia coli*, *Listeria monocytogenes, Salmonella* Typhimurium, *Salmonella* Enteritidis, *Staphylococcus aureus*, and *Yersinia enterocolitica* (Buzrul & Alpas, 2007; Chen & Hoover, 2004; Fernández, Collado, Cunha, Ocio, & Martínez, 2002; Fernández, Ocio, Rodrigo, Rodrigo, & Martínez, 1996; Fernández, Salmerón, Fernández, & Martínez, 1999; Fernández, López, Bernardo, Condón, & Raso, 2007; González, Skandamis, & Hänninen, 2009; Juneja, Cadavez, Gonzales-Barron, & Mukhopadhyay, 2015; Juneja, Cadavez, Gonzales-Barron, Mukhopadhyay, & Friedman, 2016; Mafart, Couvert, Gaillard, & Leguerinel, 2002; Peleg & Cole, 1998; Virto, Sanz, Álvarez, Condón, & Raso, 2005; Villa-Rojas et al., 2013).

2.3 Nonisothermal model

For nonisothermal conditions, that is, when the temperature or other lethal agent varies over time, a methodology for estimating survival parameters was proposed by Peleg and Penchina (2000). The authors assumed that the momentary inactivation rate in nonisothermal heat treatments is that which corresponds to the momentary temperature $T(t)$, in the time corresponding to the momentary survival ratio $\log S(t)$ (Peleg & Penchina, 2000; Peleg, Normand, & Corradini, 2005). In other words, the survival or inactivation curve $\log S(t)$ has a slope at a given time t corresponding to the slope of an isothermal survival curve at the momentary temperature $T(t)$, i.e. $-b(T)\, n(T)\, t^{*n(T)-1}$ at a momentary time t^* wich corresponds to the momentary survival ratio $\log S(t)$, i.e. $t^* = \left(\frac{-log\ S(t)}{b(T(t))}\right)^{\frac{n(T)-1}{n(T)}}$ (Peleg, Penchina, & Cole, 2001). Simply put, at very small time periods, the change (slope/derivative) of the survival curve at a momentary time can be happening at isothermal conditions. The proposed nonisothermal inactivation rate model can be calculated as (Peleg & Penchina, 2000):

$$\frac{\mathrm{d}\log S(t)}{dt} = -b[T(t)]\, n[T(t)] \left(\frac{-log\, S(t)}{b(T(t))}\right)^{\frac{n[T(t)]-1}{n[T(t)]}} \quad (17)$$

For nonisothermal treatments, the integration of Eq. (17) up to a processing time t results in the survival of the microbial population for that given time. The solution of this equation can be obtained by numerical integration, for example using Mathematica® or by programming a numerical integration routine in a computing language such as MATLAB® (Campanella, 2016). The boundary condition to solve this equation is specified at $t = 0$ $\log S(0) = 0$ (Peleg & Normand, 2004). In addition, if the temperature history $T(t)$is known, it can be inserted in the differential equation, Eq. (17), along with the $b[T(t)]$ and $n[T(t)]$ relationships, to calculate the microbial survival curve, $log\, S(t)$ vs. time (Peleg et al., 2001; Campanella, 2016). The applicability of the nonisothermal inactivation rate model has been demonstrated with experimental data of *Salmonella* Typhimurium (Mattick, Legan, Humphrey, & Peleg, 2001), experimental data of a cocktail containing *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Copenhagen, *Salmonella* Montevideo, *Salmonella* Thompson and *Salmonella* Heidelberg (Takhar, Head, Hendrix, & Smith, 2009) and published data of *Listeria monocytogenes* (Peleg et al., 2001).

The next step is to define the $b(T)$ and $n(T)$ functions. It was found empirically that in the lethal range, $n(T)$ has a weak relation with temperature or with other lethal agents (Chen & Hoover, 2004; Chen, Campanella, & Barbosa-Cánovas, 2012; Fernández et al., 2002) and, therefore, it can be assumed to be constant (Peleg & Normand, 2004) or it can be an optimized parameter obtained from the numerical interactions after solving Eq.(17). As explained by Peleg (2006), the parameter $b(T)$ reflects the steepness of the survival curve thus it can be considered as a non-linear rate constant or as the rate of killing. Typically, the rate of killing is a function of the treatment temperature. To describe such relationship the function $b(T)$ could be, for instance, taken as an analogy with a chemical reaction and, therefore, be represented by the Arrhenius equation:

$$k(T) = A\, e^{\frac{-E_a}{RT}} \quad (18)$$

A is the frequency factor (also called the pre-exponential factor), E_a is the activation energy, R is the ideal gas constant, and T is the absolute temperature in Kelvin degrees. This equation was originally formulated to describe the effect of temperature on the rate of chemical reactions in

which the covered temperature range is very large. For chemical reactions, the equation can be represented by the logarithm of the reaction rate k and the reciprocal of the temperature expressed in Kelvin, T_k. Thus, the plot Ln $k(T)$ vs. $1/(T)$ (T in°K) describes a linear relationship between these terms. However, this linear relationship may not be a true representation of food systems. For example, the quality loss of a food at 15°C is different than that at 25°C but, if the quality loss is assumed to follow Arrhenius approach, those temperatures would be converted to 0.00335 and 0.00347°K^{-1}; an interval of values so small that give a misleading impression that there may be no significant difference of their impact. Likewise, the effect of temperature on the inactivation of spores, either at 120°C or at 110°C, requires different process times. But if inactivation is assumed to follow the Arrhenius eq., then these temperatures would be converted to the indistinguishable 0.00261 and 0.00245 °K^{-1}, which leads to the erroneous supposition that there is not much difference between those process temperature (Peleg, Normand, & Corradini, 2012).

To differentiate the inactivation patterns at different process temperatures, an alternative model for calculating the effect of temperature on the inactivation rate is the log-logistic equation:

$$b(T) = Ln\,\{1 + \exp[k\,(T - T_c)] \qquad (19)$$

T_c is the critical temperature where inactivation intensifies, k represents the rate that $b(T)$ increases with the temperature after the critical temperature is exceeded. The almost perfect fit of the log-logistic equation to describe the inactivation data as function of temperature was first proposed by Campanella and Peleg (2001) when modeling the thermal inactivation of *Clostridium botulinum* spores. Later, the suitability of the Eq. (19) inserted in Eq. (17) has been demonstrated with published data of *E. coli* (Corradini & Peleg, 2004), *Salmonella* (Peleg & Normand, 2004), and experimental data of *Bacillus sporothermodurans* (Periago et al., 2004). The main advantages of assuming that the inactivation rate follows Eq. (19), rather than the traditional Arrhenius eq., are that it does not imply that the destructive effect of heat is the same either at low or high temperatures and that, consequently, there is a qualitative difference between lethal and non-lethal temperatures (Corradini & Peleg, 2004; Peleg et al., 2005). For this reason, this equation was chosen in this dissertation to account for the lethal effects when modeling survival curves. The inadequacy of using the Arrhenius equation for predictive purposes is shown in Chapter 4.

2.4 Microwave heating: current models used for modeling inactivation curves

Microwave heating is a technology that is gaining popularity in the food processing industry due to its wide applications including, but no limited to, pasteurization, sterilization, cooking, baking, and drying (Kumar & Sandeep, 2014). Recent reviews of the diverse portfolio of the application of microwave heating, and the use of the technology to assist other food processing interventions can be found in the literature (Chandrasekaran, Ramanathan, & Basak, 2013; Chizoba Ekezie, Sun, Han, & Cheng, 2017; Salazar-González, San Martín-González, López-Malo, & Sosa-Morales, 2012). It is quite likely that the use of microwave heating as an alternative to conventional heating will become more prevalent in the next decade (Kumar & Sandeep, 2014). Consequently, mathematical models for describing microbial inactivation will be required to design adequate microwave treatments.

As in conventional thermal processes, time and temperature are the main parameters used to calculate thermal inactivation kinetics. Only a few researchers attempted to model microbial inactivation under microwave heating, the few who tried, mostly used the same first-order kinetics model.

Tajchakavit, Ramaswamy, and Fustier (1999) processed apple juice under continuous microwave conditions (700W, 2450MHz) to the selected outlet temperatures of 52.5–65°C. Juices were inoculated with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*, and the destruction of these spoilage microorganisms was described using first-order kinetics. The authors calculated the D and z values of both microorganims and compared their values with conventional heating using a water bath at the same selected temperatures. There was a lot of differences between results. For example, the D-value for *Saccharomyces cerevisiae* at 55°C under microwave heating was 2.1s, while in the water bath heating it was 25s. The calculated z value were 13.4 and 15.9°C for *S. cerevisiae* and *Lactobacillus plantarum*, suggesting that the former is more sensitive to temperature changes than the later. The authors argueed that these values could be strain specific, specific to the heat treatment conditions or the media used. However, what is intriguing about this study is that the authors clearly obtained downward concave curves, and yet first order kinetics were used to model the survival curves. Therefore, the calculation of D and z-values would make the results highly questionable.

Cañumir, Celis, Bruijin, and Vidal (2002) also explored first order models to describe the pasteurization of apple juice using microwave heating to inactivate nonpathogenic *E.coli* at the

power levels 270W, 450W, 720W, and 900W using a home 2450MHz microwave. The reported outlet temperatures were, respectively, 38, 58.5, 76.2, and 70.3°C. The calculated D-values were 3.88, 0.99, 0.48, 0.42 min, respectively. The reported z-value was 58.5°C, which is a extremely high value. The z-value, for instance, of *Escherichia coli* O157:H7 in heat treated apple juice in the range 52-58°C is 4.8°C (Splittstoesser et al., 1996). Yet, the survival curves, particularly for higher powers such as 720W and 900W, which achieved almost 6 log reductions, were downwardly concave, questioning what is the usefulness of assuming linear modeling for these cases? Why is the D-value even calculated when the actual survival data from which it is derived are not really log linear?

Gentry and Roberts (2005) pasteurized apple juice at 71°C for 6s using a continuous-flow microwave system at 2000W (2450MHz). The authors calculated the lethality following Eq. (11) using T_{ref} equal 80°C and z-value equal 6°C. By assuming a $D_{80°C}$ equal to 0.755s, the authors calculated a thermal death time (F-value) following Eq. (13) and found the value of 3.9s. From that equation, the theoretical log reduction was calculated, and resulted in a 5.13 $\log_{10}$ reduction. This calculated value agrees with the reported experimental inactivation reduction. However, the reference temperature of 80°C, the z-value, and D-value chosen appear to have been purposely selected at specific values, so that the calculated and the experimental log reduction could match. Therefore, calculating the survival ratio using this arbitrary reference temperature becomes ambiguous and questionable at best.

Benlloch-Tinoco et al. (2014) also used first-order kinetic models to describe the inactivation kinetics of *Listeria monocytogenes* in kiwifruit puree under power levels 600W, 900W, and 1000W with processing times ranging from 50 to 340s. The authors calculated $D_{60°C}$ values for each power level, resulting, respectively, in the values 42.85s, 17.35 and 17.04s. When calculating the F-value, the maximum temperature reached during each microwave process was considered as T_{ref}. As discussed earlier, the selection of these reference temperatures appears to be completely arbitrary, since no reasoning for doing those selections are discussed on these articles. For historical reasons, industrial microbiologists feel more comfortable calculating the efficacy of the heat treatment using an arbitrary reference temperature so that the treatment can theoretically be related to an isothermal process at that temperature (Peleg, 2006). However, microwave heating is far from isothermal. So, what is the point of calculating the

lethality of the process and relating it to an isothermal process? Even when the fundamentals of ${F^z}_{ref}$ are questionable in the first place?

Valero, Cejudo, and García-Gimeno (2014) used two modeling approaches, the 'log linear + shoulder' model, and the modeling approach by Mattick et al. (2001), to describe the inactivation kinetics of *Salmonella* Enteritidis in potato omelet under power levels of 300W, 450W, 600W, and 800W. The inactivation curves showed a downward concave shape, so it makes sense to describe these curves with models different than first-oder kinetics. The 'log linear + shoulder' model was described as:

$$N = N_0\ e^{-k_{max}t}\ \frac{e^{k_{max}}\ Sl}{1 + (e^{k_{max}Sl} - 1)e^{-k_{max}t}} \tag{20}$$

N is the cell concentration (CFU/mL) after a treatment time t (s), N_0 is the initial cell concentration (CFU/g), k_{max} is the maximum inactivation rate (s^{-1}), and Sl is the shoulder length (s) (i.e. the length of the lag phase) (Valero et al., 2014). The objective was to fit the data and estimate k_{max} and Sl. The use of Mattick approach consisted of using the non-linear model, Eq. (17), and the $b(T)$ and $n(T)$ functions defined as:

$$b(T) = 6.841/\{1 + \exp\,[(76.14 - T)/4.204]\} \tag{21}$$

$$n(T) = 0.670/\{1 + \exp\,[(T - 80.14)/3.785]\} \tag{22}$$

Eq. (17) was solved numerically using the fourth-order Runge-Kutta method (Valero et al., 2014). According to the authors, a better agreement between experimental and predicted values was obtained when the Mattick approach was used. The modeling approach shows merit due to the good agreement between predicted and observed values, and also because the authors used a nonisothermal modeling approach to describe the inactivation kinetics of *Salmonella* Enteritidis under microwave heating, which makes perfect sense. Yet, the authors did not validate the modeling approach at other microwave conditions.

The 'log-linear + shoulder' model, Eq.(20), and the Weibull model, Eq.(15), were used to describe the inactivation curves of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in peanut butter treated by a 915 MHz microwave oven specified at 2, 4, and 6kW power levels, for up to 5 min (Song & Kang, 2016). According to the authors, both models described the survival curves of these pathogens, with the 'log linear + shoulder' model resulting in the best fit to the data. Although this article has shown improvement in using non-linear models to describe the inactivation curves, the potential relationship between temperature

changes and microbial inactivation was completely ignored. Thus, it is questioned what is the utility of such a modeling approach to optimize the microwave heating pasteurization process for peanut butter when a realistic and omnipresent condition of microwave processing is ignored in the optimization process?

More recently, Siguemoto, Gut, Martinez, and Rodrigo (2018) described the inactivation kinetics of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in apple juice treated with microwave heating at power levels 400, 600, 800 and 1000W at heating times between 50 and 390s, using a 2450MHz microwave oven adapted with fiber-optic temperature probes. The authors used the Weibull model to describe the survival curves. The Weibull parameters were calculated for conventional heating using a water bath set at temperatures 55, 60, 65 and 70°C. The Weibull scale parameter was related to the temperature using the Bigelow model. The z-value was calculated for each microorganism at a reference temperature of 70°C. Based on the integral form of the Peleg's nonisothermal model, the predicted survival ratio was calculated in which the microwave temperature history and the Weibull parameters obtained under conventional heating were incorporated. According to the authors, this approach showed a good agreement between predicted and experimental values. This study showed an improvement in the development of models for microwave heating by incorporating the dynamic microwave temperature profile. However, the calculation of the survival ratio was still related to a first-order kinetics and the z-value, which seriously challenge the suitability of the approach, mainly because the survival curves all desviated from linearity. In addition, no validation analysis was performed.

Clearly, all studies that attempted to model microbial inactivation under microwave heating presented problems associated with their methodologies. In some cases, either the survival curves were assumed to be linear, although they were clearly non-linear, or the approach did not incorporate the microwave temperature profile, neither the resulting model was validated using other samples or conditions such as power levels. These limitations open the opportunity for the development of more rigorous models that could relate microbial inactivation with the temperature profile during the microwave heating. To fill this gap, this dissertation proposes a modeling approach based on the omnipresent nonisothermal condition in microwave processing to describe the inactivation kinetics of pathogens of concern at temperatures and process times similar to those of conventional pasteurization protocols. The reader is referred to Chapter 4 to see more details of this work.

2.5 Pulsed Electric Fields (PEF): current models used for modeling inactivation curves

Pulsed Electric Field (PEF) processing is one appealing novel technologies for food preservation (Altunakar & Barbosa-Cánovas, 2011). The technology is considered non-thermal because inactivation of spoilage and pathogenic microorganisms occur at comparatively low to moderate temperatures (50–60°C) which makes it a promising alternative to traditional pasteurization methods (Arroyo & Lyng, 2016; Buckow, Ng, & Toepfl, 2013; Saldaña, Puértolas, Monfort, Raso, & Álvarez, 2011). Most of the studies available for food treatment have reported the use of microseconds or milliseconds pulse duration, and field strength of 20−90kV/cm (Liang, Mittal, & Griffiths, 2002).

Various models have been proposed to describe the kinetics of microbial inactivation by PEF. To highlight a few studies, Álvarez, Mañas, Condón, and Raso (2003a) used the Weibull distribution to model the inactivation of *Salmonella* Enteritidis and *Salmonella* Typhimurium with electric field strengths from 5.5 to 28 kV/cm, square-waves of 2µs width, pulse frequency of 1Hz, using a PEF system consisting of electrodes with area 2.01cm^2 and 0.25cm gap distance. The obtained survival curves did not show a linear behavior and thus, the Weibull distribution was used to estimate the inactivation parameters. The authors reported that the scale parameter decreased when the electric field strength applied increased. The shape parameter varied randomly and not as a function of the electric field strength. For both serovars, there was a linear relationship between log_{10} of b and the electric field strength in the range of 19 to 28 kV/cm (Table 2). From these linear equations, the authors calculated the inverse of the slope of the regression line as a way of estimating a parameter like the z value, which the authors called as Z_{PEF}. A tertiary model was then built based on the Weibull distribution and on the calculated Z_{PEF} (Table 2). Results indicated that the proposed tertiary model reasonably described the inactivation kinetics of *Salmonella* Enteritidis and *Salmonella* Typhimurium by PEF in the range of 19 to 28 kV/cm of electric field strengths. The article has merit by using non-linear models, such as the Weibull model, when clearly the survival curves deviate from linearity. On the negative side, the reason why the article brings the Z value of the linear thermal models for the PEF field is unclear. The reason for a linear relationship between the log of the parameter b and the strength of the electrical field would be indicating that the range of electrical strength used was small, and that the resistance of the microbial population to inactivation by the electrical field is constant, so the presence of a critical value for the electrical strength is ignored. In addition, the fact that an arbitrary electric field

strength could be selected to be any value within the range investigated makes the approach questionable at best. Ultimately, the incorporation of the so-called Z_{PEF} value into the Weibull model does not make much sense.

Álvarez, Virto, Raso, and Condón (2003b) studied the inactivation of *E. coli* K-12 inoculated in citrate-phosphate buffer subjected to an electric field strength in the range 15-28 kV/cm, square waves of 2μs pulse width, pulse frequency of 1 Hz and electrodes with an area of 2.01 cm^2. The survival curves were concave upwards; thus, the curves did not present a linear behavior. To describe the survival curves, four mathematical models were employed and compared: a two-term exponential model, the Weibull model, a sigmoidal equation, and an empirical equation. The four models are summarized in Table 2 along with a description of model parameters. According to the authors, the selected models were able to fit the experimental data and to describe the non-linear behavior of survival curves of *E. coli* processed by PEF in the entire range of electric field intensities investigated. In addition, the Weibull model was reported to better fit the survival curves obtained at 15 and 19kV/cm. Secondary models were also proposed in which the electric field strength was correlated to the model parameters (Table 2). The developed modeling approach has its significance, but it has not proven to accommodate the effect of the environmental conditions as the authors claimed it did. Factors like pulse width, pulse repetition rate, conductivity and residence time, for instance, were not considered in the modeling equations.

Rodrigo, Barbosa-Cánovas, Martínez, and Rodrigo (2003) investigated the PEF inactivation kinetics of *E. coli* in orange juice mixed with carrot juice. The electric field strengths ranged from 25 to 40 kV/cm, applied between 40 to 340μs in bipolar square-waves of 4μs width using the OSU-4D bench-scale continuous PEF system. The survival curves were fitted to THE Bigelow, Hülsheger, and the Weibull model (Table 2). For each model, survival parameters were obtained by adjusting the experimental survival data to these mathematical models. The goodness of fitting was evaluated by the mean square error (MSE). The authors reported that the best fit was obtained by Weibull distribution (lowest MSE). The Weibull parameters were then correlated with the electric field strength and carrot juice concentration by multiple linear regression (Table 2) and fitted to the data. The authors argued that the modeling developed can help to determine the combination of electric field strength, conductivity and treatment time to maximize PEF applications. The limitation of this article is that the approach was not validated at other PEF processing conditions.

Gómez, García, Álvarez, Condón, and Raso (2005) studied the inactivation kinetics of *Listeria monocytogenes* resuspended in citrate-phosphate McIlvaine buffer with different pH and treated by PEF. *L. mono* was more sensitive to PEF in media of low pH for all the investigated electric field strengths. Survival curves were obtained by plotting the $\log_{10}$ of the survival ratio versus treatment time at different field strengths and pH. The curves were fitted by the Weibull model. A multiple linear regression model containing three predictor variables (E, E^2, pH^2) was used to describe the relationship between the scale parameter b, the electric field intensity E, and the pH of the medium. In addition, the authors described the relationship between the parameter n and the pH by the Gompertz equation. Both were introduced into the Weibull model to generate a tertiary modeling approach. The approach was tested with published data of *L. mono* in apple juice and, according to the authors, resulted in satisfactory predictions. The article presents merit by examining a non-linear model, such as the Weibull distribution, as an alternative to first-order kinetics. However, it is not clear how variables like E^2 and pH^2 could be useful in practice. This discussion was not addressed by the authors. Consequently, the lack of such a discussion does not clarify whether the proposed approach is useful.

García, Somolinos, Hassani, Álvarez, and Pagán (2009) used the Weibull distribution to model the inactivation of *E. coli* O157:H7 in apple juice by PEF during storage under refrigeration at 4°C for up to 3 days. The pathogen was inoculated in commercial apple juice and PEF treated with the electric field strengths of 17.6, 19.9, 23.1, 27.5, 30, 35, and 40kV/cm, with pulse repetition rate of 1Hz. The PEF chamber had an area of 2.01 cm^2 and 0.25 cm distance between electrodes. The scale and shape parameters of the Weibull distribution were correlated with the storage time and the electric field strength by means of multiple linear regression (Table 2). These equations were inserted into the Weibull model to obtain a tertiary equation. The authors validated this approach in another sample of apple juice sample under randomly selected treatment conditions within the original range tested. A plot of predicted versus observed values showed a moderate agreement between empirical and model values. According to the authors, this serves as proof of the validation of the modeling approach. Similarly, as discussed for the other papers, it is unclear how variables like E^2, Et or Et^2 could be useful in practice for modeling PEF processes. These issues were not addressed by the authors, thus undermining the usefulness of the approach.

Rodríguez-González, Walkling-Ribeiro, Jayaram, and Griffiths (2011) provided an additional insight on the mathematical analysis of PEF inactivation of microbial cells. *Escherichia*

coli O157:H7 cells were subjected to exponential decay shaped electric pulses of 1.5μs width, frequencies of 20 Hz, and electric field strengths between 0.8 and 2.8kV/mm. The Weibull model was used to describe the microbial inactivation under PEF. The scale parameter was correlated to temperature increase and it was assumed to follow a log-logistic equation. The log-logistic equation was inserted into the Weibull distribution, resulting in the Weibullian-Log-Logistic (WeLL) model. A description of this mathematical approach is given in Table 2. According to the authors, the approach allowed the prediction of an onset inactivation temperature (T_c) which was different for each treatment setup. For example, in the higher electric field strength, lower temperatures were required to initiate inactivation. The results indicated that the WeLL model was able to predict microbial inactivation by PEF with dynamic temperature conditions. The article brings a new way of looking at inactivation modeling by correlating the scale parameter of the Weibull model with a theoretical onset inactivation temperature and such relation described by a log-logistic equation. It is a different perspective, especially for not assuming multiple linear regression as done by previous authors. However, the authors did not validate the modeling approach with other samples or treatment conditions, neither did they include other important parameters in the modeling, such as the electric field strength and the repetition rate. Thus, there is still an opportunity to consider such parameters for modeling of PEF processes.

Zhao, Yang, Shen, Zhang, and Chen (2013) developed a modeling approach that aims to account for lethal and sublethal injury of *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* inoculated in milk after PEF processed. The applied electric field strengths ranged from 15 to 30kV/cm, using square-waves of 2μs pulse width applied from 0 to 600μs and repetition rate set at 200Hz. The percentage of injured cells was calculated based on the proportion of counts of CFU/mL in selective media or non-selective media. The Hülsheger model was used to study the inactivation kinetics. To describe the survival curves, two versions of the Hülsheger model were proposed. For a specific treatment time, the first equation assumed a linear relationship between the logarithm of the surviving fraction and the electric field intensity. From this equation, a critical electric field could be calculated. For a specific electric field intensity, a second equation related the logarithm of survivor faction to the logarithm of treatment time. From such a relationship, a critical treatment time could be calculated. A description of these modelling approaches can be seen in Table 2. The model parameters obtained from the optimization routines included E_c, t_c, k_E, and b_t. According to the authors, critical electric field strength and critical

treatment time, which caused sublethal injuries, were lower than those that caused lethal injury. The study has its merits because the critical electric field and critical time are incorporated into the modeling analysis. However, the model approach has not been validated to confirm its predictive capacity in other treatment conditions, leaving the window open for future research that can validate models that consider treatment time, and a critical electric field as one of the main parameters.

Huang, Yu, Wang, Gai, and Wang (2014) investigated the effect of electric field strength, treatment time, and specific energy on the PEF resistance of *Escherichia coli* DH5α, *Staphylococcus aureus*, *Saccharomyces cerevisiae* in grape juice. The electric field strengths used ranged from 9 to 27kV/cm using monopolar square-waves, applied between 34 to 275μs with a minimum pulse width of 2.6μs, a frequency of up to 400Hz, and juices at an initial treatment temperature of 40°C. Survival curves were modeled using Weibull distribution in terms of treatment time and specific energy. The authors reported that the logarithm of the scale parameter (referred to in this study as δ), expressed in terms of treatment time and specific energy, decreased when the electric field strength increased. A multiple linear regression equation described the relationship of scale parameter δ and the electric field strength. The shape parameter (referred to in this study as p) varied randomly with electric field strength and, consequently, was defined as its mean value for each strain. A tertiary model was developed by replacing the $log_{10}\,\delta(E)$ relationship in the Weibull model. A summary of the models used in this study can be found on Table 2. The authors argued that the Weibull model could be safe to model inactivation of the three strains PEF-treated in grape juice. However, as discussed for the other studies, one might ask how variables like E^2 and E^3 are useful practically in modeling microbial inactivation? Finally, the approach is challenged to be validated under other conditions and samples, which was not attempted by the authors.

Walter, Knight, Ng, and Buckow (2016) studied the inactivation of *E. coli* and *Pseudomonas fluorescens* in whole milk treated by PEF. The OSU-4G PEF system composed of four treatment chambers, electrodes gap distance of 3.53mm and treatment volume of 8.98μL was used in this study. Electric field strengths of 30 and 35kV/cm, pulse width set to 2μs, pulse frequency in the range of 209-626Hz, and a flow rate of 120mL/min were the PEF treatment conditions applied. In addition, the inlet temperature was adjusted for each treatment combination to obtain an outlet temperature of 30, 40, and 50°C. The goal was to investigate synergistic effects

of temperature and PEF variables in the microbial reduction. For instance, milk treated at 35kV/cm, 50µs and 50°C achieved greater than 6 log reduction of *E. coli*. Yet, when thermally treated, in a stainless-steel coil submerged in a controlled oil bath, at 56°C for 18min the *E. coli* population was only reduced by 1.2 log reduction. Survival curves showed deviations from log-linearity, and according to the authors, the Weibull model did not yield satisfactory fits (data not shown). The curves were modeled by a log-logistic equation which the authors called a log-decay model. The coefficients of the model such as k, inactivation rate constant, and λ, decay constant, increased with increasing the temperature during thermal and PEF processes. Thus, empirical models were related to the temperature through equations that had empirical coefficients (e.g. A, B, C, and D), and a reference temperature of 50°C (T_0). These empirical models can be seen in Table 2. The modeling approach was fitted to the data by a statistical package and, according to the authors, it resulted in a satisfactory performance. Although the idea of incorporating the temperature into the modeling of PEF inactivation kinetics is valid, the authors failed to explain the reason why the temperature of 50°C was chosen. Besides, the usefulness of the empirical coefficients A, B, C, and D was completely ignored. The lack of these discussions shows that the selection of such temperature or coefficients were rather arbitrary. Moreover, clearly there was a significant effect of the PEF parameters that yielded a 6-log reduction of *E. coli*, but yet, these parameters were not included in the developed modeling approach. There is no reason to believe that the approach would be useful to illustrate the influence of the temperature on the effectiveness of PEF processes, as claimed by the authors. Lastly, no validation of the approach was attempted which leaves room for the development of predictive modeling in PEF processes that can be validated in other treatment conditions and samples.

Following the interests of the PEF in the dairy industry, Simonis et al. (2019) studied the inactivation of *Saccharomyces* sp. and *Lactobacillus* sp. in acid whey concentrate by PEF treatments with electric field strengths of 39, 95, and 92kV/cm, pulse duration of 60, 90 and $1000ns$ and number of pulses of up to 100 pulses. Acid whey, or sour whey, is a byproduct of the cottage cheese or strained yogurt manufacture processes which contains several bioactive compounds of interests for the management of human diseases such as hypertension and cardiovascular diseases. The limitation is that this product may have its useful life reduced by spoilage microorganisms. In this study, two PEF apparatuses were used to inactivate spoilage microorganisms, and the resulting survival curves were modeled. The stationary apparatus

consisted of cuvettes with cylindric stainless steel electrodes with a diameter of 5.3mm and gap distance of 0.75mm, exposed to square-wave monopolar pulses with a duration of 60 and $90ns$. Samples were treated with electric field strengths 92 and 95kV/cm. The other apparatus consisted of cuvettes connected to a peristaltic pump at a flow rate of 2mL/min. Electrodes area of $130mm^2$, 2mm gap distance and a volume of 0.26mL were used to treat the samples. A field strength of 39kV/cm of a unipolar exponential damped pulse of $1\mu s$ and pulse number of up to 32 pulses were applied. The survival curves the curves were plotted as a function of the number of pulses (pn). The Weibull model was used to model the non-linear characteristics of the curves. Like previous studies, the shape of the curves was properly described by the Weibull model. However, there were many modeling opportunities that the authors could have explored in this article. For instance, the different pulse widths (micro or nanoseconds) could have been used in modeling the inactivation curves, along with the treatment residence time and the electric field strengths. Yet, none of these parameters were discussed or accounted into the model. This highlights the need for models that are more practical in the design of PEF treatments. Treatments that have a vast range of applications, such as obtaining spoilage-free acid whey, could benefit from modeling strategies to optimize the PEF processes.

Another recent article, using the Weibull model to describe inactivation curves, investigated the effect of PEF on the inactivation of *Acetobacter* sp. cultured in samples containing different concentrations of ethanol. *Acetobacter* sp. is a group of spoilage microorganisms that negatively affect the quality of wine. Because thermal processes can damage the flavor, taste, and color of wines, PEF provides an opportunity to avoid these deleterious effects, resulting in treatment temperatures lower than those of traditional thermal methods. Niu et al. (2019) investigated PEF treated *Acetobacter* sp. cultivated in different ethanol concentration to access the effect of different ethanol concentrations on the resistance of *Acetobacter* to PEF. Cells were suspended in sterile distilled water and treated by a PEF system described elsewhere. Bipolar square wave of 40μs with electric field strengths of 10, 15, 20 and 25kV/cm and pulse frequency of 1kHz were applied. The Weibull model accurately described the shape of the survival curves. However, this study could have contributed more to understanding of the kinetics of inactivation of *Acetobacter* sp. if processes parameters such as electric field strength, repetition rate, pulse width, and residence time were accounted for in the model. It is important to describe the shape of

the inactivation curves, but still the objectives of the mathematical models are not fully explored if they are limited only to modeling a concave up or down shape.

An overview of current applications and new opportunities for the use of PEF in the food industry can be found in Barba et al. (2015). Buckow et al. (2013) also presented an overview of the application of PEF processing to orange juice and its impact on microbial, enzymatic, nutritional and quality attributes. A complete review of the kinetic models used for describing the inactivation of microorganisms and enzymes exposed to PEF can be found in Huang, Tian, Gai, and Wang (2012).

Mathematical modeling is an important tool for optimizing processing parameters. To establish PEF processing protocols that provide safe or spoilage-free products, inactivation models are indispensable. Therefore, there is an urgent need to develop reliable mathematical models that accurately describe the inactivation kinetics of microbial populations in real food systems but, that also consider the wide range of PEF parameters in the modeling. Different authors have proposed the use of the Weibull model to describe survival curves, and most of these studies used multiple linear regression to correlate the electric field strength with the scale parameter. In some cases, pH and concentration were also factors incorporated in the models by means of multiple linear equations. Such approaches help to understand how different variables can maximize PEF treatment, and for the most part the authors were able to describe the shape of the survival curves by the Weibullian shape parameter. This model represents a great improvement in quantitative microbiology as compared to the use of the conventional first-order kinetic model in which data is forced through a straight line, which as a result can lead to a number of under or over-processing problems. Despite the important advances made by the Weibull model, to date no study has considered practical PEF parameters being incorporated at the same time in the description of inactivation curves. To fulfill this gap, this dissertation is proposing a modeling approach that does just that. The reader is directed to Chapter 5 for a full description of this study.

Table 2. Models used to describe microbial inactivation under Pulsed Electric Fields

Primary Model	Secondary Model	Description of parameters	Reference
Bigelow $log\, S(t) = -\frac{t}{D}$	____	$S(t)$: survival fraction t : treatment time (μs) D: decimal reduction time (min)	Rodrigo et al., 2003
Empirical model $log_{10}\, S(t) = -aLn(1+ct)$	$c = 0.0005E^2 - 0.0007E + 0.0003$	$S(t)$: survival fraction t : treatment time (μs) a : scale parameters c : shape parameters E: electric field strength (kV/cm)	Álvarez et al., 2003b
Sigmoidal equation $CFU(t) = CFU(0) \cdot \left(1 + e^{(t-m)/s^2}\right)^{-1}$	$m = 0.006E^2 - 0.370E + 6.521$	$CFU(t)$: concentration of survivors $CFU(0)$: initial concentration of the population t : $Log_{10}$of the treatment time (μs) m : $Log_{10}$of the time necessary to destroy the 50% of the population (μs) s : parameter proportional to the standard deviation of	Álvarez et al., 2003b

Table 2. Continued

		the PEF resistance($\mu s^{0.5}$)	
Two-term exponential model $S(t) = pe^{-k_1 t} + (1-p)e^{-k_2 t}$	____	$S(t)$: fraction of total survivors t : treatment time (μs) p : fraction of survivors in population 1 $1-p$: fraction of survivors in population 2 k_1: specific death rate of subpopulation 1 k_2 : specific death rate of subpopulation 2	Álvarez et al., 2003b
Weibull distribution $log_{10}\, S(t) = -\left(\frac{1}{2.303}\right)\left(\frac{t}{b}\right)^n$	$log_{10}\, b = -0.058E + 2.040$	$S(t)$: survival fraction t : treatment time (μs) b : scale parameters n : shape parameters	Álvarez et al., 2003a
	$Log_{10}\, b = 130.68E^{-1.576}$		Álvarez et al., 2003b

Table 2. Continued

$ln\, S(t) = -\left(\frac{t}{a}\right)^n$	$ln(a) = 8.46 + 0.019\, x\, (\%\, carrot\ juice) + 0.0003\, x\, (\%\, carrot\ juice)^2 - 0.29\, x\, E + 0.0026\, x\, E^2$ $n = 0.003\, x\, (\%\, carrot\ juice) + 0.53$	$S(t)$: survival fraction t : treatment time (μs) a : scale parameters n : shape parameters	Rodrigo et al., 2003
$log_{10}\, S(t) = -\left(\frac{1}{2.303}\right)\left(\frac{t}{b}\right)^n$	$log_{10}\, b = 4.82 - 0.37E + 0.0056\, E^2 + 0.63\, pH^2$ $n = 0.38 + 63.33\, e^{-e^{(-0.25\, pH + 3.37)}}$	$S(t)$: survival fraction t : treatment time (μs) b : scale parameters n : shape parameters E: electric field strength (kV/cm)	Gómez, et al. 2005
$log_{10}\, S(x) = -\left(\frac{x}{a}\right)^n$	$log_{10}\, b = 8.09 - 0.38E + 0.006\, E^2 - 0.003Et + 0.00003\, Et^2$ $n = 0.68 - 0.40\, e^{-e^{[-1.22(t - 5.55)]}}$	$S(x)$: survival fraction x : number of pulses b : scale parameters n : shape parameters	García et al., 2009
$log_{10}\, S[(t)] = -b(T)\, t^m$	$b(T) = \ln[1 + e^{-[k\, (T - T_c)]}]$	b: inactivation rate $(\mu s)^{-1}$ t : treatment time (μs) m: shape parameter k: rate T: temperature (°C) T_c: temperature level of inactivation onset (°C)	Rodríguez-González et al., 2011

Table 2. Continued

$log_{10} S(x) = -\left(\frac{x}{\delta}\right)^{p}$	$log_{10}(\delta(E)) = A_0 + A_1E + A_2E^2 + A_3E^3$	$S(x)$: survival fraction x: treatment time (μs) or specific energy (kJ/kg) p : shape parameter δ: scale parameter	Huang et al., 2014
$LR = -log\left(\frac{N}{N_0}\right)$ $LR = \left(\frac{pn}{pn_{red1}}\right)^{p}$	____	LR: log reduction N: number of colony forming units in treated suspension N_0: number of colony forming units in untreated suspension pn_{red1}: pulse number required viable cell count reduction by one log pn: pulse number p: shape parameter	Simonis et al., 2019
$log_{10}(N/N_0) = -\left(\frac{x}{a}\right)^{b}$	____	N: number of survivors after treatment N_0: number of survivors before treatment x: electric field strength (kV/cm) or treatment time (ms) a: scale parameter b: shape parameter	Niu et al., 2019

Table 2. Continued

Hülsheger $ln(S) = -b\,(\ln t - \ln t_c)$	____	b: regression coefficient t: treatment time (μs) t_c: critical time (the longest treatment for which the survival fraction is 1)	Rodrigo et al., 2003
$ln(S) = -k_E(E - E_c)$ $ln(S) = -b_t\,ln(t/t_c)$	____	S: ration between the number of survivors and the number of initial microorganisms after PEF treatment E: electric field strength (kV/cm) E_c: critical electric field strength t: treatment time (μs) k_E: regression coefficient (μs^{-1}) b_t: independent constant	Zhao et al., 2013

Table 2. Continued

Log decay model	$log_{10}\, S(t) = -\frac{k\, t}{1 + \lambda\, t}$	$ln(k) = A + B\,(T - T_0)$ $ln(\lambda) = \frac{1}{C + D\,(T - T_0) + G\,(T - T_0)^2}$	$S(t)$: survival fraction k: inactivation rate constant (s^{-1}) λ: decay constant (s^{-1}) T_0: reference temperature at 50°C T: temperatures 30, 40, 50°C t: treatment time (μs)	Walter et al., 2016

2.6 Cold plasma: current models used for modeling inactivation curves

Cold plasma is a relatively new technology included among all emerging non-thermal technologies (Mandal, Singh, & Singh, 2018). Cold plasma is referred to the fourth state of matter that is produced by excitation of gas molecules through exposure to electrical discharges (Pasquali et al., 2016). It is a partially or fully ionized mixture of gases containing reactive gas species such as positive and negative ions, electrons, free radicals, excited or non-excited gas molecules, and photons (Mandal et al., 2018).

Non-thermal plasma induced by electrical discharges is of great interest for application in the food industry because of its potential relevance for processing food at low temperatures (Chizoba Ekezie, Sun, & Cheng, 2017). When cold plasma is generated, most of the energy applied is focused on electrons rather than heating the gas stream. Consequently, the gas molecules remain around the ambient temperature (Mandal et al., 2018). Although there is no convention with respect to the temperature range, typically plasma sources operating below 60°C can be considered as cold plasma (Misra, Yepez, Xu, & Keener, 2019). The technology offers the advantages of minimal water usage, low operating temperatures, minimal destruction of nutrients or loss of sensorial properties, inactivation of pathogenic and spoilage microorganisms, including the eradication of bacterial biofilms and the inactivation of spores (Chizoba Ekezie et al., 2017; Hertwig, Reineke, Ehlbeck, Knorr, & Schlüter 2015; Mahnot, Mahanta, Keener, & Misra, 2019; Tseng, Abramzon, Jackson, & Lin, 2012; Ziuzina, Han, Cullen, & Bourke, 2015).

Main cold plasma processing parameters include the concentration of reactive species, the treatment time, the input powers and the mode of exposure (Liao et al., 2018). For example, the type and the concentration of the gas employed determines what reactive species will be produced and their amount. The exposure model, whether direct or indirect, is also a factor that influences the efficiency of cold plasma (Chizoba Ekezie et al., 2017). Generally, direct exposure leads to faster inactivation than indirect exposure (Patil et al., 2014). For the technology to be adopted in food processing, it is essential that the inactivation kinetics be quantitatively based and that the treatment times necessary to eliminate harmful and resistant microorganisms are accurately predicted.

Becker et al. (2005) investigated the inactivation of *Bacillus subtilis* sand *Bacillus stearothermophilus* under capillary plasma electrode discharge using various gas mixtures (pure He, He—N_2, He—air, dry air and humid air), as well as the inactivation of *Chromobacterium*

violaceum biofilm-forming cells exposed to an atmospheric plasma jet generated by using He flow in conjunction with N_2 gas flow. The study has relevance because spore-forming bacteria, particularly of the *Bacillus* genera, exhibit resistance to common treatments in the food industry. In the same way, biofilms negatively impact industrial processes and have undesirable effects on the safety of the process and the final product. The use of cold plasma provides an opportunity to remove or destroy these resistant organisms. However, in terms of modeling, this study has not advanced considerably. The authors used first-order kinetics to model the inactivation curves of the *Bacillus* genera and calculated D-values with the hope of facilitating comparison within this genus (Table 3). While for the biofilm inactivation curve, exhibiting a clear upward concave curve, no modeling strategy was attempted. The assumption of first-order kinetics is a serious shortcoming because it assumes that all spores are alike and that under certain lethal condition, they would all die or be inactivated at the same time; which obviously is not the case. A quick research in the literature (Setlow & Setlow, 1993, 1996) would give a glimpse of the several factors that can affect spore resistance. These factors suggest that the assumption of a spectrum of resistance within the spore population is more plausible than that of instantaneous mortality.

There are about five possible physical mechanisms that can cause plasma microbial inactivation, including reactive species, charged particles, electric field, heat and UV photons. Deng, Shi and Kong (2006) differentiated the role of each of these mechanisms and concluded that the reactive oxygen species are dominant in the inactivation of *Bacillus subtilis* spores. The results showed that oxygen atoms, metastable oxygen molecules, ozone and OH were highly bactericidal, while other charged particles, electric field, and UV photons made minor contributions in the inactivation. The primary mechanisms of inactivation were related to leakage of the contents of cytoplasm and the complete rupture of the spore membrane. Likewise, Tseng et al. (2012) also reported that plasmas had a primary effect on damage to spores' coat/inner membrane, which lead to subsequent spore leakage and inactivation. However, the focus of this dissertation is on inactivation models and their value for designing food processes, the mechanisms of inactivation of spores and their biophysics should not concern us here, therefore, the subject will not be further discussed. The reader is referred to the literature for more information on the topic (Deng et al., 2006; Tseng et al., 2012).

Perni, Deng, Shama, and Kong (2006) employed the Baranyi model, Weibull model, and an empirical model based on a third order polynomial to describe published inactivation data of

Bacillus subtilis spores in non-thermal plasmas (Table 3). The shape of the survival curves showed a deviation from linearity that the authors called as an inverse-S shape. The R^2 was used to assess the adequacy of fit, and according to authors, the three models were adequate to fit the survival curves. The article has the value of extending the application of the Weibull and Baranyi models to non-thermal plasmas, however, it does not seem to go beyond a fitting exercise. From a practical standpoint, the proposed models were not correlated with plasma reactive species. Consequently, the use of these models for the design of real plasma processes seems to be very restricted.

Roth, Feichtinger, and Hertel (2010) fitted the inactivation of *Bacillus subtilis* spores undergoing low-pressure nitrogen-oxygen cold plasma treatment to a biphasic inactivation model (Table 3). The inactivation curves were upwardly concave with a rapid inactivation phase followed by a slow inactivation phase. From the biphasic model two constants were calculated, k_1 and k_2 which translated the rate of inactivation for the two distinct phases. D-values were estimated from these rates by $D_1 = \ln 10/(k_1)$ and $D_2 = \ln 10/(k_2)$. According to the authors, the biphasic model was well suited to represent the experimental data. However, the approach of calculating two rate constants and the resulting D-values does not seem to be practical for industrial application of plasma sterilization. In addition, no validation analysis was performed to confirm the "suitability" of the approach to other plasma conditions or exposure time. Therefore, the lack of validation opens the opportunity for the development of a modelling approach that can be validated under other cold plasma conditions and samples.

Liang, Zheng, and Ye (2012) compared models to describe the survival curves of *Penicillium expansum* exposed to low-temperature plasma. The authors used first-order kinetics, Weibull and the simplified Baranyi model to fit the *Penicillium* inactivation process (Table 3). Among the models, the first-order model was chosen as the most appropriate one based on the calculation of the coefficient of determination. However, of the nine plasma treatments, only for two of them the coefficient of determination was greater than those of the Weibull and Baranyi models. Therefore, the article has conceptual errors that lead to erroneous conclusions. It seems that the authors oversight the meaning of the coefficient of determination and inferred conclusions from them related to the fitting approach. In addition, the survival curves are clearly non-linear, but, still, the author assumed them to be linear. Subsequently, a model correlating the D-value with the plasma discharge-power (*P*) and air-flow (*F*) was constructed based on the response surface methodology. Needless to say, that parameters like (*P x F*) do not provide much physical

information on the reason of their relevance or a physical meaning of their effects on microbial inactivation.

Lee, Kim, Chung and Min (2015) investigated the effects of cold plasma to inactivate, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium in cabbage, lettuce and figs. The initial plasma-forming gas was composed of N_2, or a He—O_2 mixture. *L. monocytogenes* was chosen as the model microorganism. The effect of treatment power (400, 474, 650, 826, 900W) and time (1.0, 2.3, 5.5, 8.8, 10min) on the inactivation of *L. monocytogenes* in cabbage was investigated using modeling and response surface analysis. The experimental data were fitted to the first-order model, the Fermi's kinetic model, and the Weibull model (Table 3). When power was assumed as the key variable, the treatment time was set at 5.5 min. On the other hand, when time was assumed as the key variable, the power was set at 650W. The modeling results suggest that when power was the key variable of the process, the models did not fit the data closely as they did when time was selected as the key variable. According to the authors, both first-order and the Weibull models were adequate to describe the data, while little was said about the modeling ability of Fermi's model. The study is relevant in investigating the inactivation of important foodborne pathogens that were linked to outbreaks of fresh produce in the U.S. However, it did not provide a full modeling approach. The authors mentioned that the reactive nitrogen species, and the reactive oxygen species such as O^{2+}, O_2^-, O, O_3 can damage cellular structures, lipids, membranes and DNA, however these important lethal agents were not considered into the modeling. Consequently, there are opportunities to consider these gas species in the modeling of microbial death exposed to cold plasma treatments.

Ren-Wu et al. (2015) studied effects of plasma created with He gas in the inactivation of *E. coli* and found that bacterial cells were completely killed in 60s. The modeling of *E. coli* inactivation was also attempted in this work. The authors began the modeling analysis by inferring that the inactivation process was similar to a chemical reaction process, and that one *E. coli* cell would be killed by αROS (Reactive Oxygen Species) (Table 3). The ROS concentration was considered constant, and the modeling approach was reduced to a first order kinetics. However, this assumption represents a serious flaw. The literature has reported that the concentration of reactive species actually increases with plasma processing time (Patil et al., 2014). Therefore, considering a constant concentration of ROS so that the modeling approach can be simplified is a serious shortcoming. Furthermore, the outcomes of such oversimplification would not accurately

describe the inactivation of bacterial cells and, in some situations, the method would lead to under processing and, as a consequence, lead to an increased risk. Therefore, the modeling approach attempted in this work may have a little impact in industrial applications.

Recently, Hertwig, Reineke, Rauh, and Schlüter (2017) studied the factors involved in the wild-type and mutant *Bacillus* spore's resistance to cold atmospheric pressure plasma. Different process gasses, such as dry air, N_2, O_2, and CO_2 were used as the starting gas to generate the reactive species. The cold plasma generated from O_2, and CO_2 had nearly no UV ligh emission, but generated high concentration of reactive oxygen species like ozone. The high concentration of ozone was considered the cause of the greater inactivation of certain type of *Bacillus subtillis* strains treated with O_2-generated cold plasma. The inactivation curves were non-linear and the Weibull model was used to describe the curves (Table 3). Based on the statistical indices, such as Adjusted R^2 and RMSE, the authors concluded that the inactivation kinetics were adequately described by the Weibull model. The article provides a good study on different strains of *Bacillus subtilis* spores and their inactivation under a variety of cold plasma gases. Although the authors showed higher correlation between ozone concentration and spore inactivation, this relationship was not included in the modeling analysis. Instead, the Weibull model was fitted to the data solely to confirm its ability to describe the curvature of the survival curves. Nevertheless, the lack of a model that relates the dynamic formation of reactive species like ozone to the non-linear inactivation curves of *Bacillus subtilis* spores evidences the need for modeling approaches that are practical and therefore useful for industrial applications. Chapter 3 of this dissertation proposes a modeling approach that fills this gap in cold plasma sterilization research.

Table 3. Models used to describe microbial inactivation under Cold Plasma

Primary Model	Description of parameters	Reference
Bigelow $log\, S(t) = -\frac{t}{D}$	$S(t)$: survival fraction t : treatment time (s) D: decimal reduction time (s)	Becker et al., 2005
Empirical model $log_{10}\, N(t) = \; y = at^3 + bt^2 + ct + d$	$N(t)$: spore concentration at time t a, b, c, d: fitting parameters	Perni et al., 2006
Baranyi model $N(t) = \; N_0 e^{-k_{max} t} \left(\frac{1 + \; C_c(0)}{1 + \; C_c(0) e^{-k_{max} t}}\right)$	N_0: initial bacterial concentration k_{max} : maximum inactivation constant C_c: "critical component" which may or may not be a real substance $C_c(0)$: initial concentration of the critical component	Perni et al., 2006
Simplified Baranyi $N = m \text{ x } N_0 + (1 - m) N_0 \text{ x } 10^{-bt}$	N: cell concentration at time t N_0: initial cell concentration m: moduli b: moduli	Liang et al., 2012

Table 3. Continued

First-order kinetics		
$\log N = \log N_0 - bt$	N: cell concentration at time t N_0: initial cell concentration b: modulus that characterizes the inactivation intensity	Liang et al., 2012
$ln(RM) = -k_1 P$ $\ln(RM) = -k_2\, t$	RM: residual microbial concentration k_1: first-order kinetic constant P: treatment power (W) k_2: first-order kinetic constant t: treatment time (min)	Lee et al., 2015
$\alpha\mathrm{ROS} + E.coli(\mathrm{alive}) \rightarrow E.coli(\mathrm{dead})$ $\frac{\mathrm{d}\, C(t)}{\mathrm{dt}} = -k_0 C_{ROS}^{\alpha}\, C(t)$ $ln\left(\frac{C(t)}{C_0}\right) = -k_0 C_{ROS}^{\alpha}\, t = -k_0'\, t$	$C(t)$: *E.coli* concentration at the treatment time (t) k_0: rate constant of reaction C_{ROS}^{α}: concentration of reactive species assumed to be constant k_0': constant	Ren-Wu et al., 2015
Fermi's kinetic model		
$RM = \frac{1}{1 + \exp\left(\frac{P - P_h}{a}\right)}$	RM: residual microbial P: power (W) P_h: treatment power (W)	Lee et al., 2015

Table 3. Continued

	a: parameter indicating the slop of the curve around P_h	
Biphasic inactivation model $N/N_0 = fe^{-k_1 t} + (1-f)e^{-k_2 t}$	N: number of survivors N_0: number of initial population t : treatment time (min) f : constant designating the transition from the first phase to the second k_1 : inactivation rate for the first phase k_2 : inactivation rate for the second phase	Roth et al., 2010
Weibull model $N(t) = N_0\ 10^{-(t/\delta)^p}$	$N(t)$: spore concentration at time t N_0: initial bacterial concentration δ : time required to reduce the viability of the cell population by 90% p: shape parameter	Perni et al., 2006
$\log(-N/N_0) = \log b + n log\ t$	N: cell concentration at time t N_0: initial cell concentration b: moduli n: moduli	Liang et al., 2012

Table 3. Continued

$\log N = \log N_0 - \left(\frac{t}{\delta}\right)^s$	N: number of survivors N_0: number of initial population t: treatment time (min) s : shape parameter δ: scale parameter	Lee et al., 2015
$\log S(t) = - b\ t^n$	$S(t)$: survival fraction t : treatment time (min) b : scale parameter n : shape parameter	Hertwig et al., 2017

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CHAPTER 3. MODELING THE INACTIVATION OF *BACILLUS SUBTILIS* SPORES DURING COLD PLASMA STERILIZATION

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3.1 Abstract

Cold plasma sterilization is an emerging non-thermal technology that is receiving great attention in the food processing area. Plasma is a neutral ionized gas composed of reactive gas species that inactivate bacteria or spores in a variety of food materials without compromising the main physico-chemical characteristics of the food. Survival curves of *Bacillus subtilis* spores were obtained after spore strips samples containing am initial spore population of 1.5-2.5 x 10^6 cfu/strip were subjected to plasma treatment. The shape of the survival curves was clearly not linear indicating that spores exhibit a spectrum of inactivation resistances to the plasma treatment. A Weibull model was used to describe these curves. In order to capture the effects of the typical variability in the concentration of the inactivating reactive gas species during plasma processing, time-varying concentrations were incorporated in the calculating approach. The result was an ordinary differential equation (ODE) that was numerically solved using MATLAB. This approach was successfully applied to describe the survival of *Bacillus subtilis* spores during plasma processing as well as data obtained from the literature for *B. atrophaeus*. Ozone was assumed the lethal reactive gas species responsible for spore inactivation. Modeling plasma processing is of great interest because it may provide an accurate estimation of time and conditions required for a complete plasma-based sterilization process.

Key words: *Bacillus subtilis*; cold plasma; survival curve; Weibull model; spore inactivation; non-thermal technology

3.2 Introduction

Cold plasma is a non-thermal process that is receiving great attention as an innovative emerging technology to sterilize foods without changing their main physical properties and composition (Li & Farid, 2016; van Bokhorst-van de Veen et al., 2014). It has been used to inactivate pathogens and spores of public health concern, such as *Salmonella spp.*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Kim et al., 2015; Lee, Kim, Chung, & Min, 2015; Pasquali et al., 2016; Ziuzina, Patil, Cullen, Keener, & Bourke, 2014; Ziuzina, Han, Cullen, & Bourke, 2015). Patil et al. (2014) achieved inactivation of *Bacillus atrophaeus* inside of sealed packages, showing the suitability of the process to treat foods packaged in materials sensitive to temperature (van Bokhorst-van de Veen et al., 2014). Inactivation of endogenous enzymes has also shown the potential of cold plasma to ensure enzymatic stability while retaining initial product quality (Bußler, Steins, Ehlbeck, & Schlüter, 2015; Tappi et al., 2015). Non-food applications include sterilization of medical instruments that are sensitive to heating (Patil et al., 2014; Venezia, Orrico, Houston, Yin, & Naumova, 2008). Recently, the uses of cold plasma processing in a number of applications have been discussed in several reviews (Li & Farid, 2016; Misra, Tiwari, Raghavarao, & Cullen, 2011; Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015) in a number of applications.

Plasma is defined as a partially or fully ionized gas composed of photons, ions, and free electrons as well as atoms in their fundamental or excited states having a net neutral charge (Misra, et al., 2011; Moreau, Orange, & Feuilloley, 2008). Two classes of plasma, namely thermal and non-thermal, are defined depending on the conditions they are generated. Thermal plasmas are obtained at high pressure and require higher power (up to 50 MW) to be created (Fridman, Gutsol, & Cho, 2007; Moreau et al., 2008). They can be found in plasma torches and electric arcs, for instance. In contrast, non-thermal plasmas are generated in lower pressure and require lower power (Moreau et al., 2008). Nonthermal plasmas can be generated by dielectric discharges in low-pressure gases or using microwaves. Plasma generated at atmospheric pressure, and with a temperature close to ambient, is of technical and industrial interests because they do not impose extreme conditions to the product (Misra et al., 2011; Moreau et al., 2008). A variety of terms can be used to describe plasmas of this type including atmospheric pressure plasma, cool plasma, cold plasma, among other comparable terms (Niemira, 2012). Cold plasma discharges can be produced by a variety of means including dielectric barrier discharges (DBD), the corona discharge, radio

frequency plasma, and the gliding arc discharge (Fridman et al., 2007; Misra et al., 2011). A discussion on the various cold plasma techniques, their use for decontamination or sterilization, their properties and limitations can be found on the literature (Moreau et al., 2008; Niemira, 2012, 2014). For decontamination and sterilization purposes of nonthermal plasmas in contact with liquids, a useful reference is the review of Bruggeman and Leys (2009), which emphasizes the generation mechanisms and physical characteristics of these plasmas in a great detail.

Dielectric barrier discharges (DBD) is one of the most convenient ways to generate plasmas (Ziuzina, Patil, Cullen, Keener, & Bourke, 2013). This type of discharges has a broad range of industrial applications because they operate at non-equilibrium conditions at atmospheric pressure and at reasonably high power levels without using sophisticated pulsed power supplies (Fridman et al., 2007; Moreau et al., 2008). Through the flexibility of DBD configurations in terms of the electrode geometrical shape and the dielectric material employed, optimized laboratory scale experiments can be scaled up to large industrials installations (Kogelschatz, Eliasson, & Egli, 1997; Ziuzina et al., 2013). For these reasons, DBD discharges were employed in this study to produce cold plasma.

The cold plasma generated through a dielectric barrier discharge burns in the presence of natural or synthetic air, plus other gases such as nitrogen, oxygen, hydrogen, argon or their mixtures (Scholtz et al., 2015). The gases that pass through the discharge are ionized by high-energy electrons accelerated in an electrical field (Misra et al., 2011) and the result is a fully or partially ionized mixture of reactive gas species capable of killing bacteria or inactivating spores (Venezia et al., 2008; Ziuzina et al., 2013). The reactive species generated include reactive oxygen species (ROS), reactive nitrogen species (RNS), energetic ions, ultraviolet (UV) radiation, and charged particles. It has been revealed that the concentration of reactive gas species increases significantly with plasma exposure time. Keener et al. (2012) and Patil et al. (2014), for instance, have shown the increase in ozone concentration for different plasma sterilization treatments.

Traditionally, the inactivation of microbial and spore cells has been assumed to follow first-order kinetics, and this model has been used to quantify risk assessments in food safety (Gomez-Jodar, Ros-Chumillas, & Palop, 2016). The approach, required by regulatory agencies, is used in the canning industry to predict the effects of different processing conditions on the survivability of lethal anaerobic microorganisms such as *Clostridium botulinum*. The assumption of first-order kinetics to describe the inactivation reaction yields log-linear survival curves that are used to

predict the inactivation of an organism exposed to given processing conditions (Chen, Campanella, & Corvalan, 2007). Although first-order kinetics have been extensively used, many survival curves reported in the literature have shown nonlinear relationships with either downward or upward concavity (Campanella, 2016; Van Boekel, 2002). Thus, for these cases, the first-order kinetics fails to describe the mortality of microbial cells. Therefore, a model considering different shapes of microbial inactivation should be used (Aspridou & Koutsoumanis, 2015; Campanella & Peleg, 2001; Chen et al., 2007; Peleg & Penchina, 2000; Peleg, Normand, & Campanella, 2003). Furthermore, the first order kinetics does not take into account the heterogeneity of cell communities as a source of variability in population dynamics (Koutsoumanis & Aspridou, 2016), and assumes that microorganisms have identical resistance to the lethal agent, which does not appear to be a realistic assumption. Some microorganisms are inactivated sooner or later than others, thus showing a spectrum of resistance in their population (Peleg & Cole, 1998). The different spectrum of resistances of the microbial population to an inactivation process leads to the conclusion that the mortality of microorganisms is ultimately a random process (Fredrickson, 1966); and the shape of the survival curves is determined by the microorganisms' distribution of resistance to the treatment. More specifically, a probabilistic model examines shapes of mortality curves that are in relation to the statistical properties of the distribution of resistances of the individual cells or spores to the lethal agents (Aspridou & Koutsoumanis, 2015; Peleg & Cole, 1998). A number of non-linear models have successfully described the shapes of mortality curves or inactivation curves, and one of the more commonly used is the Weibull distribution (Mafart, Couvert, Gaillard, & Leguerinel, 2001; Peleg & Cole, 1998; Peleg & Penchina, 2000; Peleg & Normand, 2004; Van Boekel, 2002).

The Weibull model was selected in this work to describe the inactivation parameters from experimental data of *B. subtilis* spores subjected to cold plasma sterilization. In order to capture the varying conditions of ozone concentration, these temporal variations were incorporated in the model to calculate the microorganism's inactivation during the process. This approach was first proposed by Peleg et al. (2003) and was applied to thermal sterilization. In this work, the approach is extended for application to cold plasma sterilization. To test the applicability of the approach in plasma processing, *B. subtilis* spores were used as the target microorganism because it is a heat-resistant spore that often compromises the thermal efficacy of heat processes (Gomez-Jodar, Ros-Chumillas, & Palop, 2016). There are multiple mechanisms involved in spore resistance that

appear to be conserved across *Bacillus* species, and most of the studies on mechanisms of spore resistance have used *B. subtilis* spores (Setlow, 2006). *B. subtilis* is a non-pathogenic bacterium that has been extensively used as surrogate microorganism for pathogenic bacteria. When direct measurements are feasible, the use of the surrogate microorganisms of high resistance are often used; for instance, for parasites such as *Giardia lamblia* and *Cryptosporidium parvum* (Yousefzadeh et al., 2018) as well as surrogates for bioterrorism agents like *Bacillus anthracis* (DeQueiroz & Day, 2008). Besides their use as surrogates for a broad range of microorganism, the ease of count and identification of these cells on laboratory conditions has contributed significantly to the decision of selecting these spores as the target for other investigations and the present study. Concerning this manuscript, once the model was developed to describe the inactivaion of the surrogate spores the approach was tested to describe the inactivation of *B. atrophaeus* spores using data found in the literature. The treatments involved cold plasma treatments with two types of sterilizing gases (air and a mixture of 90% N_2 and 10% O_2) and two exposure modes, direct and indirect exposure of samples to the plasma gases (Patil et al., 2014).

Until now, models that consider the generation of ozone over time and their relation to microbial death have not been investigated. Therefore, the aim of the present work was to develop a model able to assess how dynamically changing ozone concentration affect the inactivation of microbial cells during plasma treatment. The development of a suitable model has remarkable industrial relevance because it contributes to accurately predict the treatment time necessary to achieve a safe reduction in microbial viability using real processing conditions.

3.3 Material and methods

3.3.1 log-lineal or first-order kinetics

The assumption of first-order kinetics to a plasma process yields, upon integration, the following equation:

$$N\,(t) = N_0\,\exp(-k't\,) \qquad (1)$$

N(t) is the number of microorganisms or spores alive after treatment of time *t* with a certain plasma gas concentration, N_0 is the initial concentration of spores and *k'* is a rate constant. Another way of expressing Eq. (1) is by defining the survival ratio or spore reduction $S(t) = N(t)/N_0$ and after rearrangements of Eq. (1) the following equation is obtained:

$$\log S(t) = -\frac{1}{D}t = -b\,t \tag{2}$$

Eq. (2) is an alternative way of expressing the microbial survival as a function of an exponential distribution function. The exponential distribution function is one of the many parametric models used for analyzing survival data. However, this type of distribution assumes that the hazard rate b is constant, which implies that the probability of microbial death remains constant as the plasma treatment progresses. It also implies that the probability of microbial depends exclusively on the processing time considered (Machin, Cheung, & Parmar, 2006, chap.4) at a specific processing condition. The hazard rate b is also a function of the lethal agent, thus the fact that the concentrations of the reactive species are not constant during plasma processing invalidates the assumption that the parameter remains constant during the process. Moreover, when the hazard rate is considered constant, the spores have identical resistance to the lethal agent. However, studies have shown that some spores are inactivated sooner or later than others due to the existence of a spectrum of resistance to the treatment within the microbial population (Peleg & Cole, 1998). Therefore, an exponential distribution function assumed when the first order kinetics is considered would not be appropriate for analyzing spore survival data for more realistic situations.

3.3.2 The Non-Linear Survival Kinetics

A model able to describe different shapes of mortality curves is the Weibull model. The Weibull distribution assumes that the hazard rate is not constant, but rather increases or decreases with time (Machin et al., 2006 chap. 4). Perni, Deng, Shama, and Kong (2006) have shown that this model is useful to describe the inactivation kinetics of plasma processing. For constant concentration of the reactive species, it can be assumed the following format of the model (Peleg & Cole, 1998; Peleg & Normand, 2004):

$$log\, S(t) = -b\,(C)\,t^{n(C)} \tag{3}$$

where C is the concentration of the reactive species, and $b(C)$ and $n(C)$ are concentration dependent survival kinetics parameters. The shape of the survival curves is given by the parameter n. When $n>1$ the curve has a downward concavity, it is a straight line when $n=1$ (lineal case – Eq. (2)) and has an upward concavity when $n<1$ (Campanella, 2016). For the present study, n is treated as a concentration-independent parameter because its values are randomly distributed as ozone concentration changes. The coefficient $b(C)$ is the rate of killing of spores and increases with the

concentration of the reactive gas species. It can be further assumed that during the treatment no growth or recovery of spores is taking place.

The relationship between the parameter $b(C)$ and the reactive species concentration C is assumed to follow the log logistic equation (Campanella & Peleg, 2001; Peleg & Normand, 2004):

$$b\,(C) = \ln[1 + \exp\,(k(C - C_C))] \tag{4}$$

k and C_c are parameters that specifically depend on the type of spore. A critical or threshold concentration, C_C, is a concentration above which the inactivation of that microorganism or spore becomes effective, while k measures the effects of a gas specie concentration on $b(C)$ at the inactivation region. It should be noted that the above two equations imply that the concentration of reactive and inactivating species remain constant during the treatment, which is an assumption that rarely holds for real plasma treatments. Plasma gases are generated and vary over time, so a model to describe the concentration of these time varying reactive species could be used. Peleg et al. (2003) used the following model:

$$C(T) = \frac{t}{C_1 + C_2 t} \tag{5}$$

Eq. (5) could be replaced for experimental data of reactive species versus time, and an interpolation function could be used instead. Furthermore, since the concentration of reactive species generated is non-constant, it is preferable to use the local rate of change of survived spores, which can be calculated by differentiating Eq. (3) with respect to time. Peleg and Penchina (2000) proposed this methodology to estimate survival parameters under nonisothermal conditions in thermal processes by assuming that the local slope of the nonisothermal survival curve was a function of the momentary temperature $T(t)$. By applying the same assumption for plasma processing with non-constant reactive species concentrations, the corresponding survival curve of spores can be described by the differential equation:

$$\frac{\mathrm{d}\log S(t)}{dt} = -b[C(t)]\,n[C(t)]\left\{\frac{-log\,S(t)}{b(C(t))}\right\}^{\frac{n[C(t)]-1}{n[C(t)]}} \tag{6}$$

Eq. (6) can be solved using the initial condition $log\ S(t)=0$ at $t=0$. Given the non-linear characteristics of Eq. (6), it has to be numerically solved as an initial value problem using a software such as MATLAB® (Mathworks®, Natick, MA, USA) to estimate the survival curve in terms of the $log\ S(t)$ versus t, under conditions of non-constant reactive gas species concentrations.

It must be noted that the numerical approach does not provide a solution when the initial condition is used because the logarithm of S(t) =1 is not defined at the initial conditions. Thus, if the initial condition at *t=0* is replaced by a small value such as *log S(t)* = 10^{-7}, the numerical integration provides the right solution. Several small initial values were used with no changes in the result of the integration. The applicability of the approach is demonstrated with experimental inactivation data from *B. subtilis* spores assuming ozone as the leading reactive gas species responsible for spore inactivation.

3.3.3 Microbial Survival data

3.3.3.1 Spore population and sample preparation

Bacillus subtilis var. niger (*B. atrophaeus*) spore strips (NAMSA, Northwood, OH, USA) with size 3.2 cm × 0.6 cm were used. Each spore strip contained a spore population of 1.5-2.5 × 10^6 cfu/strip. Single spore strips were loaded into an open sterile polystyrene petri plate, which was then placed inside the treatment package made from a Cryovac® B2630 high barrier film (Sealed Air Corp., NC, USA). The samples were placed in the center of the electrodes and directly exposed to cold plasma.

3.3.3.2 Cold plasma treatment

Dielectric barrier discharges (DBD) plasmas were employed to inactivate *B. subtilis* spores. Fig. 3 shows a schematic view of the DBD utilized for the cold plasma treatment. Ionization electrodes consisted of rectangular wrappings of copper wire coils approximately 7.5 cm x 11.5 cm placed directly above and below the center of the treatment package. The packages (22 cm x 30 cm) were sealed, purged and filled with 1.76 l of the working gas containing 22%O_2, 30%N_2, 40%CO_2 and 8% Ar, using a calibrated flow meter. The ionization system was run at 50 kV (65-75W @ 0.5-0.8mA) with an electrode gap or depth of 2.5 cm. Samples exposures time to the cold plasma discharge were 0, 15, 30, 60, and 120s. The tests were replicated five times, and average values are reported. All treated packages were stored for 24h at room temperature (22°C), and then bacterial spore recoveries were conducted using standard microbiological methods. In addition, plates were monitored up to 72 hours post-recovery to monitor any potential regrowth from injured cells.

3.3.3.3 Spore recovery

Spore recoveries and aseptic methods were followed as indicated per manufacturer (NAMSA, Northwood, OH, USA) for the population estimation of the *B. subtilis* spore strips. After the ionization treatment and 24h storage, each strip was aseptically removed from its treatment package and transferred into sterile 20 x 150 mm test tube containing 9.6 ml of 0.1% sterile peptone. Twelve sterile 6-mm glass beads were then added to each test tube. Each test tube was vortexed (model vortexer 59, Denville Scientific, Inc., Metuchen, NJ, USA) on high speed for 2-3 min or until the spore strip was fully macerated. Test tubes were then heat shocked to fully germinate spores in a 90°C water bath and held for 10 minutes. Test tubes were transferred to a cold tap water bath for 2min, and then to ice water bath to rapidly cool the test tubes to 0-4°C. The test tubes were then removed from the ice bath, and serial dilutions were performed including 10^{-2}, 10^{-3}, 10^{-4}, and/or 10^{-5} based on the treatment or recoveries of positive (+) controls (*Bacillus* populations of 1.5-2.5 x 10^6/strip, or 6.2-6.4 log). The required aliquot volumes from the corresponding serial dilutions were then plated into respective sterile petri plates (100 x 15mm) containing sterile Tryptic Soy Agar (TSA, Becton, Dickison and Co., Sparks, MD, USA) prepared per Difco™ manual specifications for spore colony enumeration. TSA plates were incubated at 35°C and colony growth, and recoveries were monitored at 24h, 48h, and 72h.

3.3.4 Gas measurements of reactive species

The working gas, containing 22%O_2, 30%N_2, 40%CO_2 and 8% Ar, was certified by the manufacturer (People's Welding, West Lafayette, IN). The sealed packages (22 cm x 30 cm) were filled with 1.76 L of gas at a rate of 2.1 L/min using a calibrated flow meter (Model 2260, Gilmont Instruments, Inc., Barrington, IL, USA) with an average fill time of 50s. The packages were then stored at room temperature (22°C). Gas concentration in the sealed packages was verified using a calibrated oxygen analyzer (Mocon®, Model 302, Minneapolis, MN, USA). Ozone and nitrogen oxide gas concentrations were measured using the Drager® gas analysis system (Dräager Safety AG & Co., Lübeck, Germany) immediately after treatment and after 24h at room temperature storage (22°C). The system was selected for ease of use with the given experimental setup and rapid measurement capabilities. Tubes containing a colorimetric reagent, which changed color upon contact with the specified gas were calibrated for specific sampling volumes. These tubes were connected to a bellows hand pump, Drager® Accuro Gas Detector Pump (Drager Safety AG

& Co., Lübeck, Germany), and calibrated such that one pump equals 100 ml of gas. Ozone tubes (Mfr no. CH 21001) had a measured range between 20 and 300 ppm whereas the measurement range for nitrogen oxide (Mfr no. CH 24001) was 20-500 ppm. A cross-sensitivity of 5 ppm NOx per 100 ppm ozone was identified and corrected for in reported values. Carbon monoxide tubes (Mfr no. CH 33051) had a measurement range of 25-300 ppm. It was noted that carbon monoxide tubes had interference with ozone. Thus, carbon monoxide measurements could not be taken with ozone present. Initial concentrations of carbon monoxide were determined from measuring half-life decay at 24 hours and extrapolating to initial conditions. Measurements of reactive gas species such as ozone, nitrogen oxide, and carbon monoxide were performed, and it was found that ozone concentration was 12 and 29 times greater than the concentration of nitrogen oxide and carbon monoxide, respectively. Moreover, unlike the other gases whose concentration varied randomly, the concentration of ozone was progressively increasing with time. Thus, it was assumed that ozone was the key reactive gas promoting inactivation of the spore cells. In order to capture the steady increasing ozone concentration, this time variation of ozone concentration was incorporated in the model to estimate spores' reduction potential during the process. This approach was first proposed by Peleg and Penchina (2000) and was applied to thermal sterilization, which is extended in this manuscript to estimate the efficacy of cold plasma sterilization.

In order to determine ozone values when testing high concentrations, smaller gas sample volumes (2 or 5 ml) were collected in a 5 ml syringe. The syringe was connected to the detection tube by small (2 cm length) flexible tubing. A syringe volume was expelled into the detection tube and then removed allowing the total flow volume of 100 ml to occur. The observed gas concentration was then multiplied by the volume ratio of the detection tube volume over the syringe volume (x50 or x20). For 24-hour ozone sampling, a low concentration ozone tube (Mfr. no. 6733181) was used with a measurement range of 0.05-0.7 ppm. The Drager® portable gas detection system had a precision of ±15% (Drager Safety AG&Co., Lübeck, Germany).

3.3.5 Model development

Traditionally the parameters $b(C)$ and n, at specific concentrations, are estimated by fitting the experimental survival curve with the kinetics model selected to describe the inactivation of the microorganisms. However, in these calculations is assumed that those parameters are determined under constant concentrations conditions, which are difficult to achieve in the cold plasma

treatment. Thus, the changes in the inactivating reactive gases with time have to be incorporated in the calculations. As discussed, the generation of reactive species (ozone in this case) can be properly described by Eq. (5). This expression is empirical and itself irrelevant as long as it can ably describe the reactive gas species concentration as a function of time and be able to be incorporated into Eq. (6) along with Eq. (4), which in this manuscript describes how the Weibull parameters are affected by the concentration of the reactive species.

Given the non-linear characteristics of the ordinary differential equation (ODE), the solution of Eq. (6) along with Eqs. (4) and (5), requires a numerical approach such as the Runge-Kutta method that is implemented in software like MATLAB®, used in this research. The Runge-Kutta method was deemed to be appropriate for these calculations because it is suitable to solve ODE when moderate accuracy ($\leq 10^{-5}$) is required (Press, Teukolsky, Vetterling, & Flannery, 1992, chap. 16). Specifically, the ordinary differential equation solver *ode45* was used in the solution of Eq. (6), where the effect of the concentration of reactive species C on the kinetics parameters b is described by Eq. (4) whereas the time dependence of the ozone concentration $C(t)$ by Eq. (5). The *ode45* tool was selected because includes a variable time step allowing for efficient computation (Bober, Tsai, & Masory, 2010, chap. 6). The solution of Eq. (6), with $C(t)$ described by Eq. (5), can be used to adjust the inactivation parameters k, C_C, and n that better describe the experimental data by minimizing the SSE determined from the experimental data and the solution of the model. A Matlab script was written and a special search was utilized to find the set of parameters that minimized the square deviation between the values of the microbial population estimated from the model and the mean value of the microbial population at a processing time. Survival population at times 0s, 15s, 30s, 60s and 120s were recorded, and the corresponding SSE values were determined. The global minimum SSE for the whole process was obtained from the SSE obtained at each time interval. For further analysis, the RMSE value was defined as:

$$RMSE = \sqrt{\frac{SSE}{m-p}} \tag{7}$$

where m is the number of observations and p is the number of parameters estimated.

3.4 Results and discussion

3.4.1 Model application on *B. subtilis* data

3.4.1.1 Traditional Approach – calculations based on the concentration of inactivating gas constant

The inactivation curve of *B. subtilis* spores resulting from plasma treatment is shown in Fig. 4. This figure clearly shows that the inactivation of spores does not follow first-order kinetics and is clearly not linear; it also displays a concave upward shape. The results indicate a microbial population reduction greater than six logarithmic cycles in viable cell concentration after approximately 120 seconds of plasma treatment, corroborating the efficacy of the sterilization treatment.

A typical fitting approach of the experimental data using Excel™ was performed to obtain the survival kinetics parameters defined by Eq. (3). Average parameter values estimated from five replicates are shown in Table 4.

Table 4. Survival parameters estimated from the Weibull model assuming constant concentration conditions:

Parameter	Estimate
n	0.142
b	0.808

It is important to note that these parameters are obtained with the assumption that the concentration of the reactive lethal gas remains constant during the process. However, this is not the case for cold plasma processing (refer to Fig. 5). Thus, the Weibull model parameters given in Table 4 do not provide a true representation of the survival kinetics because they ignore that the concentration of ozone is varying with time. Despite the incorrect assumption, a reasonable fit of the Weibull model to *B. subtilis* spore's survival data is observed in Fig. 4, with a RMSE of 0.76. Concerning the shape of the survival curve, if profoundly scrutinized, it can be observed that the Weibull model described it as an upward concavity (i.e., $n < 1$ see Table 1). However, it does not appear to be the case, as it can be clearly seen a shoulder at t = 15s with a downward concavity after that time. About this, a survival curve showing a shoulder should yield the shape parameter greater than 1 (i.e. ,$n > 1$) revealing a downward concavity shape. This highlights the inherent

difficulties in attempting to use this model for cold plasma processing. It also suggests that due to the expected varying concentration of reactive gases over time in the treatment, this condition should be incorporated into the modeling approach. In fact, the initial low concentration of the reactive gases in the beginning of the treatment was probably not sufficient to inactive the spores, but once this concentration reached the critical or threshold value, spores were inactivated at a higher rate exhibiting a downward concave shape ($n > 1$). Indeed, application of the classical approach assuming constant concentration of the reactive gas using a linear kinetics, Weibull, or any other kinetics would lead to incorrect information.

The reactive gases species ozone, nitrogen oxide, and carbon monoxide yielded maximum concentrations of 5900 ppm, 485 ppm and 200 ppm respectively [data not shown]. By considering the highest concentration generated corresponded to ozone, this gas was considered as the major lethal agent in the process. This assumption appears to be solid and supported for practical experience because ozone, which is included in the ROS group, is considered one of the most important antimicrobial agents playing a key role in the inactivation of spores (Lu, Patil, Keener, Cullen, & Bourke, 2014; Misra et al., 2011). In order to describe the variation of ozone concentration with time during the process, the experimental data was fitted with Eq. (5). Results are illustrated in Fig. 5, where a $R^2 = 99.4\%$ indicates an excellent fit with model parameters C_1 and C_2 yielding values 0.0043 and 0.0006, respectively. The shortcoming in using an empirical model is that it is not related to the plasma chemistry. Relationships between the plasma chemistry and the concentration of the reactive lethal gas is a subject currently under study. Although not mathematically/physically rigorous, the proposed empirical model for ozone concentration reproduced the ozone generation fairly well, as observed in Fig. 5. Further developments could potentially relate these empirical model parameters to physical features of ozone generation from plasma processing, and with other reactive gas species that may play a role in spore inactivation.

3.4.1.2 Modeling Approach – calculations based on time-varying concentration of inactivating gases

To address the difficulties in applying the Weibull model for a constant concentration of the principal reactive lethal agent, the model described as Eq. (6), which includes a variable concentration of ozone. The speed and convergence of the solutions depend on the specified range of initial guesses used for the parameters n, k, and C_C. In order to assess the convergence of the approach and test the result of the shape parameter on the solution, two value ranges for the

variable n were considered: [0.25–1.5] and [0.25–2.0] with six or seven values within that range, respectively. The range for k was [10^{-5} to 10^{-2}] with 1000 values considered within this range, whereas the range for C_C was [10^2 to 10^4] considering 100 values within this range. As discussed, the best estimation of the n, k, and C_C parameters was based on the minimization of the global SSE. Thus, although the computation was a formidable task, the software was able to converge to a solution in a relatively short time. The range the guesses used for the parameter n affected especially the parameter C_C; however, some values obtained for this parameter did not have physical meaning, so the corresponding range was discarded. For instance, if the range of guesses for n was [0.25–2] the obtained value for the Cc parameter was 6800, which exceeded the maximum ozone concentration of 5900pm generated in the plasma treatment (see Fig. 5). In other words, the value of the critical or threshold concentration Cc is never reached during that treatment, which under these conditions would result in no inactivation. Some of the conditions necessary to discharge some unrealistic solutions are straightforward in the approach. Peleg et al. (2003) discussed the physical meaning of the threshold parameter T_c in thermal treatments, and similar concepts apply to plasma processing.

Survival parameters estimated from the modeling approach presented in this work in which the variation of ozone concentration is being considered, as well as the corresponding RMSE value, are presented in Table 5. Besides providing more realistic values of the survival kinetics parameters, values of RMSE indicate an improvement on the goodness of fitting of the model to the experimental data. It is clear from Fig. 6 that the description of the survival curves was significantly improved by using the approach developed in this work. The major advantage of the program written in MATLAB® to solve Eq. (6) is that it can extract a set of inactivation parameters that take into account realistic scenarios in which the concentration of ozone, or in fact other reactive gases used alone or in combination, changes with time. The approach also provides a more realistic explanation of the results confirming the expectation that $n > 1$, which would lead to survival curves with downward concavity. As illustrated in Fig. 6, this result is in a better agreement with experimental data and better describe the presence of the shoulder in the survival curve. The observed initial downward concavity would be indicating that the spores are weakened by the exposure to the lethal agent, and the time needed to inactivate them is progressively shorter. A slight tail in the survival curve observed for times between 60s and 120s of treatment is also captured by the proposed model (Fig. 6). This tail most likely is showing that after 60s, the weaker

spore population is completely inactivated and it becomes more difficult to inactivate more resistant spore populations. By comparing Fig.4 and 6 is possible to see that the modeling approach was able to reproduce better the experimental survival curve of *B. subtilis* spores, and thus estimates inactivation parameters for this microorganism under more realistic processing conditions. Comparing the survival curves illustrated in Figs. 6B and C, it can be observed that results are very similar and there is only a small difference between their RMSE. Choosing between them would be a matter of checking which one would result in a more meaningful critical concentration parameter (C_C). This could be done by checking the maximum amount of ozone generated in the process and comparing that value with the value of C_C obtained from the approach. As illustrated in Fig. 5, the highest ozone concentration generated was approximately 5900 ppm, and since the critical concentration should be smaller than that value a C_C = 3600 ppm would be a more feasible value than C_C = 6800 ppm. Consequently, the best set of model parameters *n*, *k*, and C_C that realistically captures the inactivation of *B. subtilis* spores are respectively 1.5, 0.0025 ppm^{-1} and 3600 ppm.

Table 5. Estimated inactivation parameters, *n, k*, and C_C of *Bacillus subtilis* data subjected to plasma treatment under a concentration of ozone changing with time.

Method	*n*	*k* (ppm^{-1})	C_C (ppm)	RMSE
Modeling approach ozone concentration constant with time	0.81	0.0067	1800	1.05
Modeling approach ozone concentration changing with time	1.5	0.0025	3600	0.46
	1.75	0.0012	6800	0.38

3.4.1.3 Model Application to other *Bacillus* species (*Bacillus atrophaeus*)

The reduction of *B. atrophaeus* spores as a function of time for two different modes of plasma exposure (direct and indirect), and two different gas types (air and a mixture of 90%N_2 and 10% O_2) is illustrated (Fig. 7) using data from the literature (Patil et al., 2014). As observed in the figure, *B. atrophaeus* survival curves are clearly non-linear, and the inactivation of these spores certainly does not follow a first-order kinetics.

For the two different gas types, the critical concentration of ozone (Cc) was assumed equal to the maximum concentration of ozone reported by Patil et al. (2014). Application of the model to the experimental survival curves and the experimental concentration of gases versus time resulted in a set of survival kinetics parameters, which describe the experimental data through the minimization of SSE reasonably well. The obtained parameters are reported in Table 6.

Table 6. Survival parameters, *n, k*, and C_C for *Bacillus atrophaeus* obtained from the model applied to experimental data from Patil et al. (2014).

Gas Type	Mode of exposure	n	k	Cc	$\sqrt{SSE}$
Air	Direct	0.75	0.0012	5230	1.27
	Indirect	0.75	0.0012	7000	3.62
90% N_2+10% O_2	Direct	0.75	0.0021	4000	0.65
	Indirect	0.25	0.0086	4400	1.57

It is important to point out that the values of $\sqrt{SSE}$ are reported instead of the RMSE values because the number of data points are different. Some treatments had three data points and others five data points. If Eq. (7) is applied to calculate RMSE values, m=3 and p=3 for the data set of 3 points m-p is zero and RMSE cannot be determined. Therefore, $\sqrt{SSE}$ was selected to estimate the goodness of the fit of the model to describe the experimental data. Interestingly, the parameter n did not change for most of the treatments except when the treatment was indirect, and the gas was a mixture of N_2 and O_2. Moreover, if the air gas is particularly considered, it can be seen that the parameter k did not change regardless of the mode of exposure. Whether this similarity was due to the gas type and the maximum ozone generated, or to particular characteristics of the strains, it cannot be determined from the available data. However, the objective of the present work was to evaluate the feasibility of the modeling approach to estimate survival kinetic parameters of the same microorganism. Thus, this observed agreement is not of concern for the purposes of this study.

The survival parameters obtained from the solution of the model, which are reported in Table 6, were used to calculate survival curves, which are plotted along with the experimental data in Fig. 7. In Figs.7A and B, the difference in treatment times used with the two modes of exposure is because a treatment time of 60s resulted in complete inactivation of spores for direct exposure and, thus, model-based calculations were performed until that time. As illustrated in Fig. 7, the

curves closely described the data when direct plasma exposure was employed. The model was tested with data from the literature with a different strain and different treatment modes, and the goodness of the fitting was reasonable, despite the little information on the gases concentration and its dependence with time. Thus, it is expected that the modeling approach could be used for other applications of plasma processing from where a more complete data set can be obtained.

3.5 Conclusions

The work shows that experimental survival curves of *B. subtilis* spores are far from being linear and showed noticeable downward concavity when plotted in semi-logarithmic plots. The Weibull distribution was used in this research to describe the non-linear behavior of the inactivation of *B. subtilis*. However, other models could be implemented in the proposed approach without losing generality.

Regarding processing, the generation of reactive gas species during the plasma treatment is a naturally random process, and the concentration of lethal reactive gases generated is far from being constant during the process. When dealing with non-constant concentration conditions, the rate of change of survival spores is better described through a model that takes into account the change of the reactive gas (ozone in this case) concentration with time. Despite the apparent complexity of the model, equations can be solved numerically with software like MATLAB® without major difficulties. The main advantage of the approach is that it captures the inherent varying concentration conditions during plasma sterilization and, therefore, it is useful to estimate inactivation parameters of microorganisms (e.g., *Bacillus subtilis* and *Bacillus atrophaeus* spores) under more realistic processing conditions.

Admittedly, the suggested empirical model used to portray the concentration of reactive gas species as a function of time have yet to be related to plasma physics. This leaves the window open for potential future research on modeling inactivation of spores and microorganisms considering more elaborate physical models and inactivation using several reactive gases. Models of such scenarios are yet to be developed, and it is expected that the approach presented in this work will be useful for developing such models, not only to other spores and treatments but also to additional microorganisms subjected to plasma sterilization.

Author’s contribution

Gabriella Mendes Candido de Oliveira conceived the idea of this study, interpreted the data, wrote the code, ran the model, analyzed output data and wrote the manuscript. Osvaldo H. Campanella and Kevin M. Keener supervised the project. Jeanette L. Jensen performed the cold plasma experiments.

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3.6 Figures

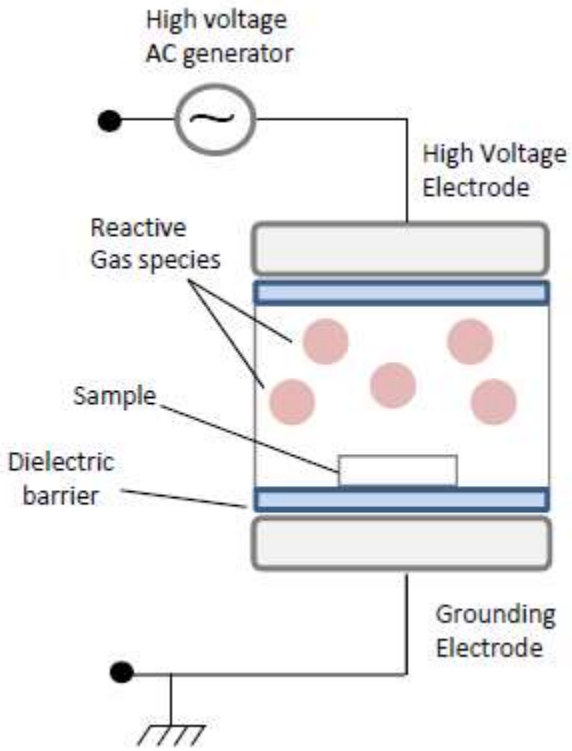

Fig. 3. Schematic diagram of the dielectric barrier discharge (DBD) used to generate cold plasma. The reactive gas species formed are illustrated as pink circles.

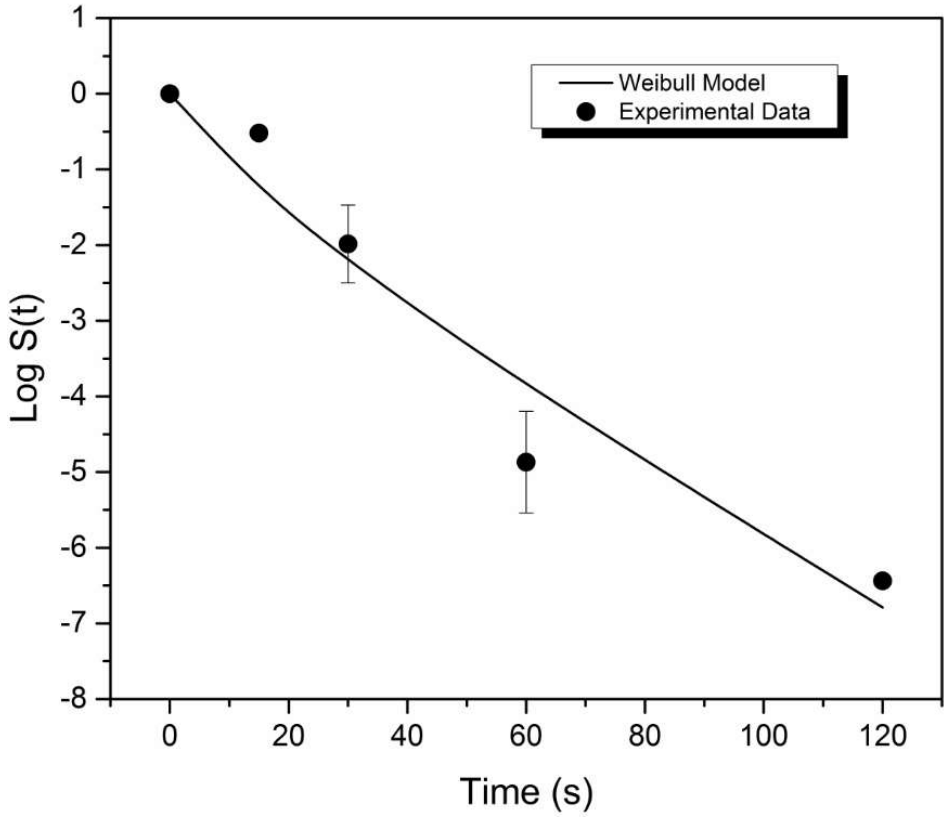

Fig. 4. Microbial inactivation curve of plasma treated *Bacillus subtilis* spores fitted with the Weibull model Eq. (3). Error bars indicate one standard error of five replicates.

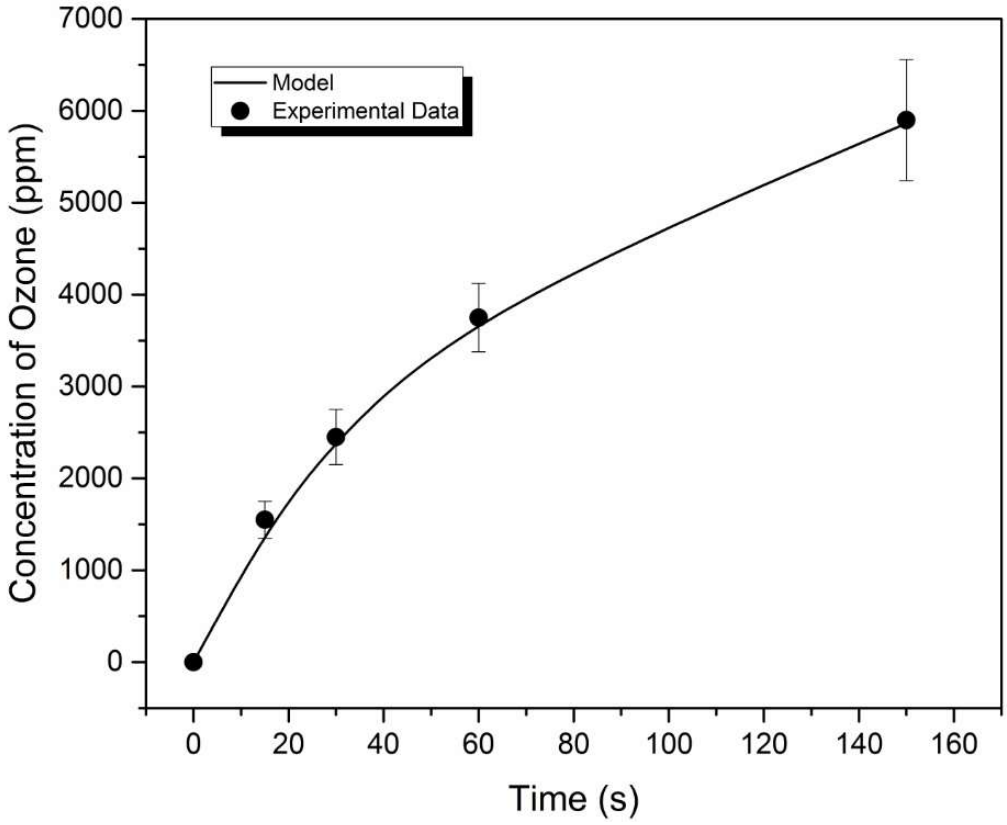

Fig. 5. Generation of ozone over time and the fit of the data with Eq. (5). Error bars indicate one standard error of five replicates.

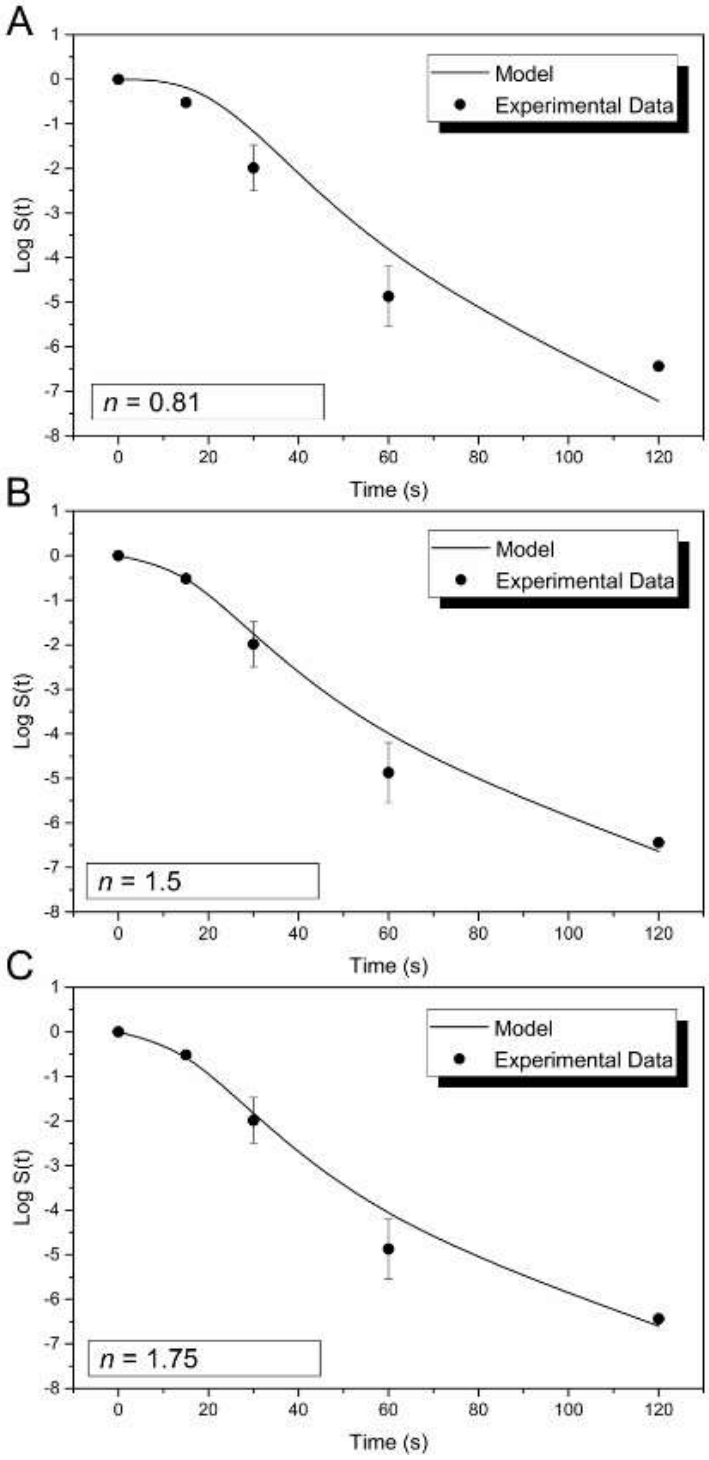

Fig. 6. Survival curve of *Bacillus subtilis* spores under plasma treatment. (A) *n* and *b* calculated by fitting experimental survival data to the Weibull Eq. (3), i.e. without considering that the reactive lethal gas is changing with time during the process. (B) and (C) experimental survival curve considering non-constant concentration conditions model expressed by Eq. (6); (B) assuming that the value of *n* is in the range 0.25-1.5. (C) assuming that the value of *n* is in the range 0.25-2.0. Error bars indicate one standard error of five replicates.

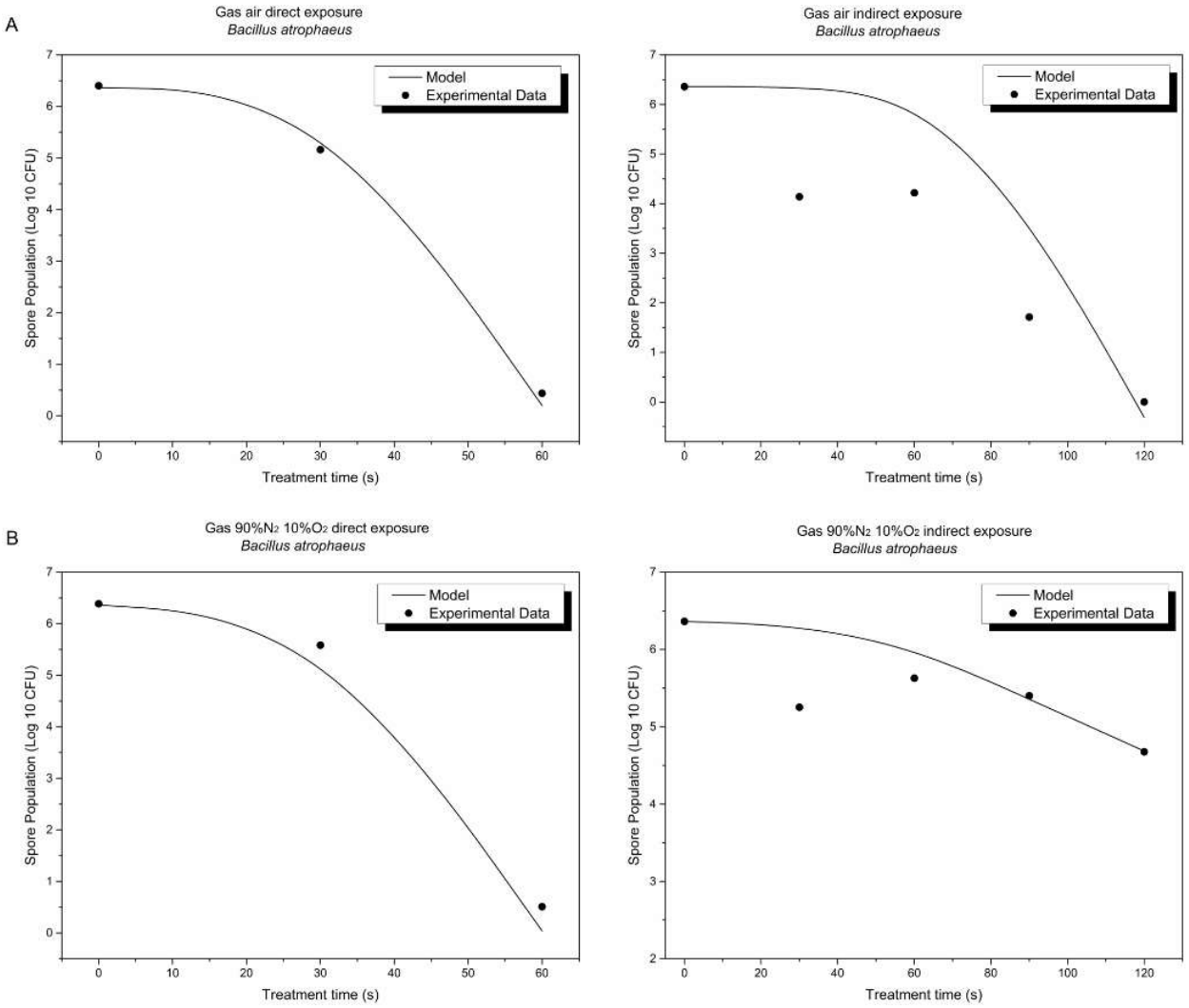

Fig. 7. The experimental reduction of spore population of *Bacillus atrophaeus* fitted with Eq. 6 (ODE Model) for different range of ozone concentration and different mode of exposure. (A) Gas type: air. (B) Gas type: 90% N_2 +10% O_2. The inactivation parameters are listed in Table 6.

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CHAPTER 4. MICROWAVE PASTEURIZATION OF APPLE JUICE: MODELING THE INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* TYPHIMURIUM AT 80-90°C

This chapter was subtmitted to the Food Microbiology Journal and is under revision

4.1 Abstract

Although due to their acidity some fruit juices are considered safe, several outbreaks have been reported. For processing fruit juices, microwave heating offers advantages such as shorter come-up time, faster and uniform heating, and energy efficiency. Thus, it could be a beneficial alternative to conventional pasteurization. The objective of this study was to study the inactivation kinetics of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium under microwave pasteurization at temperatures between 80 to 90°C, i.e., at conditions like those employed in conventional pasteurization. Inoculated juices were treated at different power levels (600W, 720W) and treatment times (5s, 10s, 15s, 20s, 25s). Time-temperature profiles were obtained by fiber-optic sensors in contact with the samples allowing continuous data collection. The log-logistic and Arrhenius equations were used to account for the impact of the temperature history; thus, yielding two modeling approaches that were compared for their prediction abilities. Survival kinetics incorporating nonisothermal conditions were described by a non-linear ordinary differential equation that was numerically solved by using the Runge-Kutta method (*ode45* in MATLAB ®). The *lsqcurvefit* function in MATLAB® was employed to estimate the corresponding survival parameters, which were obtained from freshly made apple juice, whereas the prediction ability of the obtained kinetic parameters was evaluated on commercial apple juice samples. Results indicated that inactivation increased with power level, temperature, and treatment time reaching a microbial reduction up to 7 Log_{10} cycles. The study is relevant to the food industry because it provides a quantitative tool to predict survival characteristics of pathogens at other nonisothermal processing conditions.

Keywords: Microwave, *Escherichia coli* O157:H7, *Salmonella*, mathematical modeling, Arrhenius, log-logistic equation

4.2 Introduction

The demand for fruit juices with fresh-like characteristics has substantially risen over the last years, mostly associated with the consumption of unprocessed fruit juices (Manzocco, Plazzotta, Spilimbergo, & Cristina, 2017). Due to their inherent acidity, fruit juices were historically thought to be safe (Sung, Song, Kim, Ryu, & Kang, 2014). However, several outbreaks have been associated with the consumption of unpasteurized juices contaminated with pathogens such as *Escherichia coli* O157:H7 and *Salmonella spp*. From 1995 through 2005, for instance, twenty-one outbreaks occurred in the United States due to the consumption of juice or cider (Gurtler, Rivera, Zhang, & Geveke, 2010; Vojdani, Beuchat, & Tauxe, 2008). Among these events, five of the outbreaks were caused by *Salmonella,* and five were caused by *Escherichia coli* O157:H7 (Vojdani et al., 2008).

Apple juice, which is considered one of the most popular juices in the U.S., was implicated in 10 of the 21 outbreaks reported to CDC (Vojdani et al., 2008). The emergence of juice-associated outbreaks led the FDA to issue a Hazard Analysis and Critical Control Point (HACCP) regulation on juices, which requires the process to achieve a minimum of 5-log reduction of the target pathogens of public health concern (USFDA, 2001).

Commercial heat pasteurization of apple juice typically involves temperatures of 68°C held for 20 to 30 minutes or 82°C for 20 to 30s (Zhao, Doyle, & Besser, 1993). Heating protocols involving temperatures ranging from 77-88°C for 25-30s have also been previously reported (Moyer & Aitken, 1971). Although, pasteurization at high temperatures and short times may appear as a mild heat treatment, the low penetration of the heat and the non-uniform temperatures reached in the product during conventional thermal processing may induce physical and chemical changes in the juice that result in losses of its nutritional value and organoleptic properties (Gouma, Álvarez, Condón, & Gayán, 2015; Tajchakavit, Ramaswamy, & Fustier, 1999). One way to address the challenge of retaining the quality of fresh juices that consumers are demanding is to utilize suitable alternatives to conventional thermal preservation techniques (Contreras, Rodrigo, Polite, & Kingdom, 2017). Application of microwave heating has attracted significant attention as one of the promising alternative technologies to conventional pasteurization (Contreras et al., 2017; Matsui, Granado, Oliveira, & Tadini, 2007; Tang, Hong, Inanoglu, & Liu, 2018). Microwave heating of foods differs from conventional heating due to the capacity of microwaves to penetrate into the food and dissipate energy in its interior by the interaction of the microwave radiation with

water molecules (Bhattacharya & Basak, 2017; Sumnu & Sahin, 2012). This interaction heats the food rapidly due to the internal heat generation that takes place in parallel with heat convection and conduction transfer. Conventional heating processes rely on heat transfer from external heat sources which often requires long heating times resulting in deleterious effects on the product quality and its nutritional attributes (Wang & Sun, 2012). Since the heat penetration depth of microwaves is significantly larger than that of conventional heating, the conversion of energy into heat is more efficient and uniform in the food product (Tang et al., 2018). Thus, presenting advantages such as shorter come-up time, faster heating, shorter processing time, greater energy efficiency, all resulting in products with improved nutritional and sensorial qualities (Peng et al., 2017; Salazar-González, San Martín-González, López-Malo, & Sosa-Morales, 2012). Microwave heating also offers advantages in *in-package* microwave pasteurization because reduces processing time and is suitable for pre-packaged heat sensitive multicomponent meals (Tang et al., 2018).

A discussion on the several benefits of microwave processing, its numerous advantages, applications, and effects on quality attributes of food products can be found in the literature (Bornhorst, Liu, Tang, Sablani, & Barbosa-Cánovas, 2017; Chandrasekaran, Ramanathan, & Basak, 2013; Ekezie, Sun, Han, & Cheng, 2017; Guo, Sun, Cheng, & Han, 2017; Tang et al., 2018).

In order to select appropriate processing conditions able to produce high quality and safe food products, models describing microbial inactivation kinetics are essential. Therefore, the objectives of this study were to (1) investigate the effect of microwave pasteurization on the inactivation of the target microorganisms *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in freshly prepared apple juice; (2) develop a modeling approach that incorporates the microwave temperature history in the estimation of the inactivation rate, and survival parameters calculated considering nonisothermal heating protocols; (3) compare two modeling approaches, one approach describing the effect of temperature on the inactivation rate constant by the log-logistic equation whereas the other describing this temperature dependence by the commonly employed Arrhenius equation, and (4) validate the modeling approaches and the obtained survival parameters in a commercially pasteurized apple juice inoculated with the target microorganisms and processed at other conditions (e.g. at other power level).

Specifically, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium were chosen as target microorganisms because of their potential incidence in wounded fruit or fresh-cut apple slices (Janisiewicz, Conway, Brown, Sapers, & Fratamico, 1999), and also because of the

numerous food poisoning outbreaks involving these microorganisms in apple juice (Vojdani et al., 2008). The modeling approaches were applied on freshly prepared apple juice inoculated with the target microorganisms and subjected to temperatures achieving values of 80°C and 90°C in 25s. The selection of the processing conditions was based on heating protocols used for conventional pasteurization of apple juice as described by Zhao et al. (1993), and Moyer and Aitken (1971). Survival parameters obtained from these tests were used to predict survival curves of commercial apple juice samples inoculated with the target microorganims and subjected to different heating conditions.

4.3 Materials and methods

4.3.1 Apple juice

Fresh apple juice was prepared using "Golden delicious" apples *(Malus domestica* Borkh, cv. *Golden Delicious*) purchased from a local supermarket and maintained at 4°C until use. This particular variety was selected because Golden Delicious apples are within the top 5 varieties produced in the U.S. and are frequently used to produce fresh apple juice (USAPPLE, 2016). Moreover, recent literature has also explored the applicability of alternative food technologies to process Golden Delicious fresh apple juice (Bot et al., 2017; Manzocco et al., 2017). Fresh juice was obtained using a domestic juicer (Breville 800 JEXL Juice Fountain® Elite, Breville USA Inc., Torrance, CA, United States), filtered through two layers of cheesecloth to remove impurities and coarse particles, centrifuged at 5000 rpm (1677 x g) for 5 min, and filtered again by using two layers of cheesecloth. The resulting clear apple juice having a soluble content of 12.2±0.96 °Brix and pH of 3.43±0.073 was inoculated with the targeted microorganisms and immediately treated. A fresh apple juice was prepared for every trial.

4.3.2 Defining the microwave power levels to be used in this study to achieve the targeted temperatures

Preliminary tests were conducted to determine suitable heating ranges using three key processing conditions (i.e., intensity of microwave power, final temperature reached, and treatment time) which affect the reduction of *Escherichia coli* O157:H7 on apple juice. Four power levels, 1200 W (100%), 720W (60%), 600W (50%) and 480W (40%), were applied for times ranging from 5 to 25s on 3 mL of juice inoculated with *E. coli* O157:H7. Prior to treatment, the juice had

an initial temperature of 25°C. The objective of these preliminary tests was to determine the combination of microwave processing conditions that would pasteurize the juices at temperatures ranging from 80-90°C for treatment times of up to 25s, as these conditions are reported to achieve commercial pasteurization of apple juice. After these conditions were determined, tests were conducted at the selected treatment and replicated three times at random. The electrical energy input consumed at those four power levels were also recorded using a power meter installed in the outlet where the microwave system was plugged in. The objective of this measurement was solely to assess the efficiency (power consumed/power produced) of the microwave oven used in the research. Results reported as the electrical energy input, the actual power level delivery to the food, and the final juice temperature raise by applying these power levels, as well as inactivation level of *E. coli* O157:H7 are summarized in Table 7.

Table 7. Preliminary tests and results to select suitable treatment conditions reported as energy input, power delivered, final temperature increased after 25s, and log reduction for *E. coli* O157:H7 in apple juice.

Energy input (Watts)	Power delivered (Watts) / Power Setting	Final temperature (°C)	Log_{10} reduction
1800	1200 / 100%	102	7.0
1080	720 / 60%	90	7.0
900	600 / 50%	80	6.9
720	480 / 40%	63	2.3

4.3.3 Microwave treatments

From the preliminary results (Table 7) was found that power levels of 60% (720W) and 50% (600W) would produce temperature profiles with final temperatures ranging from 80-90°C in approximately 30s. In this work, the target temperatures were achieved in 25s. For that reason, these power levels were used in the study to generate results employed in the modeling approach. Aliquots of 3 mL of inoculated apple juice samples were transferred to cylindrical glass tubes 2.5 x 5.5cm (diameter x height) placed at the center of the microwave turntable and subjected to microwave heating at two selected power levels (600W, 720W) with temperatures rising to 80°C and 90°C respectively, with a process time of 25s. A Microwave Work Station™ (Fiso

Technologies Inc., Quebec, Canada) was used in the present study. The Microwave Work Station (MWS) is an integrated system used along with FISO fiber-optics sensors which record temperatures in a customized Panasonic microwave oven (2450 MHz, 1200 W) equipped with a rotating unit, a Workstation Commander control, and data collection software. Real-time data acquisition was performed by using three FISO fiber-optic sensors directly inserted in the juice samples. Temperatures were collected every 0.45s at the selected power. Considering the sample volume used in this study and the high penetration of the microwave, it was assumed that at the end of the process the temperature was uniform within the sample.

4.3.4 Experimental design and statistical analysis

Experiments were carried out as a randomized complete block design. The inoculated juices were treated at 600W and 720W power levels at treatment times of 5s, 10s, 15s, 20s, and 25s. Microorganisms were distributed into blocks, and treatment times were randomly assigned to the experimental units within a block. All the experiments were performed independently in triplicate in different days, and the microbial analysis was performed in duplicate for each power/treatment time combination applied. Inactivation data were analyzed using the Statistical Analysis System software (SAS 9.4, SAS Institute Inc, Cary, NC, USA). The General Linear Model (GLM) module of SAS was used to evaluate treatment time and microorganism as fixed effects. Significant mean differences were calculated by Tukey's test at $p<0.05$.

4.3.5 Temperature measurement

During microwave pasteurization the temperature was measured by three FISO fiber-optics sensors (FOT-L-BA Model, Fiso Technologies Inc., Quebec, Canada) inserted inside the samples, and recorded using data collection software.

4.3.6 Apple juice soluble solids content and pH determinations

Soluble solid content (°Brix) was measured using a digital laboratory refractometer (Model LR-01, Maselli Misure, Parma, Italy). The pH was measured using a benchtop pH-meter (Thermo Scientific™ Orion™ 2- Star Benchtop pH Meter, Waltham, MA, USA) previously calibrated with standard buffer solutions.

4.3.7 Microbial growth conditions and analysis

Strains of *Escherichia coli* O157:H7 B6-914 and *Salmonella* Typhimurium ATCC 14028 were obtained from Purdue University, Center of Food Safety Engineering (West Lafayette, IN, USA). Concentrated pure strain suspensions of these bacteria were maintained at -80°C in cryovial tubes; *Escherichia coli* O157:H7 was stored as 7% dimethyl sulfoxide (DMSO) frozen stocks and *Salmonella* Typhimurium as 50% glycerol frozen stocks. Frozen stocks cultures of each strain were cultured independently in 5mL Tryptic Soy Broth (TSB) and incubated at 37°C with continuous agitation at 110 rpm for 18h. A transfer of 50µL was made into 5mL TSB and incubated at 37°C for 18h with continuous agitation (110 rpm) prior to experiments. The initial cell concentration of each bacterial culture was approximately 10^8-10^9 CFU/ml. Bacterial cultures were harvested by centrifugation at 6000 rpm (2415 x g) for 10 min, the resulting pellets were washed in 0.1M phosphate buffer (PB; 0.1M, pH 7.0) and centrifuged again. The supernatant removed, and the resulting pellets were resuspended in 1mL of 0.1M PB before inoculation. Finally, cells were inoculated in apple juice at a 1:5 ratio to obtain populations of 7-8 Log CFU/ml. Aliquots of 3 mL of inoculated apple juice samples were pasteurized by microwave heating at previously determined conditions.

4.3.8 Enumeration of survivors

Immediately after microwave treatments samples were 10-fold serially diluted in 0.9mL of sterile 0.1 M PB, and 0.1 mL of sample was spread-plated in duplicate onto selective media (Sorbitol MacConkey Agar - SMAC - for *Escherichia coli* O157:H7; Xylose Lysine Tergitol 4 Agar - XLT4 - for *Salmonella* Typhimurium). Due to dilution process the samples were rapidly cooled before plating. All dilutions were plated in duplicate and plates were incubated at 37°C for 24h before enumeration.

4.3.9 Modeling of survival curves

Survival curves were obtained by plotting the $\log_{10}$ of the survival ratio ($N(t)/N_0$) versus treatment time (s). $N(t)$ is the number of microorganisms surviving the treatment at time t (CFU/mL), and N_0 is the initial number of the microbial population.

The Weibull model (Eq. 1) has been extensively applied to describe the inactivation of microorganisms in foods over the years. The model has gained wider acceptance to study the

survival kinetics of spores and vegetative cells because of its simplicity and flexibility. It has two parameters: b, a scale parameter, and n, a dimensionless shape parameter, which are able to describe non-liner survival kinetics (Mafart, Couvert, Gaillard, & Leguerinel, 2001; Van Boekel, 2002). Considered as a primary kinetic model, is given by the following equation (Corradini, Normand, & Peleg, 2008; Peleg & Cole, 1998; Peleg, Normand, & Corradini, 2005):

$$\text{Log}\left(\frac{N(t)}{N_0}\right) = Log\ S(t) = -b\ t^n \quad (1)$$

Under true isothermal conditions, survival curves can be constructed simply by plotting the decimal $\log_{10}$ of the survival ratio versus treatment time at a given temperature. The parameters b and n can be obtained by minimization of the sum of squares error (SSE) between the model and the experimental values, using tools like *Solver or Power Trend* in Microsoft Excel™ or other statistical packages.

For nonisothermal conditions such as those occurring during microwave heating (Fig.8), the Weibull model as expressed by Eq. (1) does not account for the previous thermal history to which the population is subjected (Peleg & Penchina, 2000). Thus, the dependence of the primary model relevant parameters, e.g. *b* and *n* for the Weibull model or *k* (or D) for the first order model, with the lethal agent, temperature in this case, needs to be established. It should be noted that for non-isothermal conditions, the processing temperatures varies with time and so do the parameters of the survival model. Therefore, it is necessary to link Eq. (1) with the variation of the model survival parameters with temperature and time. Peleg and Penchina (2000) proposed a model for the calculation of survival ratio of a given microorganism under non constant processing conditions where nonisothermal conditions are clearly noticeable, which is the existing condition in the present research. The approach assumes that under nonisothermal conditions, the momentary slope of the survival curve can be considered as the change in microbial survival at that momentary temperature. In other words, it assumes that at the current processing time, the change (slope/derivative) of the survival curve at that time is happening at a isothermal condition. Therefore, the calculation of the lethality achieved during the whole process involves an integration over the range of temperatures existing during the process. This calculation is expressed by the following equation (Corradini et al., 2008; Chen, Campanella, & Corvalan, 2007; Peleg et al., 2005; Peleg & Penchina, 2000):

$$\frac{d\ log\ S(t)}{dt} = -b[T(t)]\ n[T(t)]\left(\frac{-log\ S(t)}{b(T(t))}\right)^{\frac{n[T(t)]-1}{n[T(t)]}} \quad (2)$$

It must be noted from Eq. (2) that the survival model parameters are expressed as a function of non-uniform temperature expressed as $T(t)$, which results in an non-linear ordinary differential equation that must be solved using well established numerical tools such as the Runge-Kutta method. The differential equation solver (*ode45*) in MATLAB ® (Mathworks®, Natick, MA, USA) was used to solve this equation and thus to estimate the resulting nonisothermal survival curve $log\ S(t)$ versus t under the existing processing temperature conditions. However, in order to provide a detailed description of the microbial inactivation by the solution of Eq. (2), is necessary to know how the temperature, the process lethal agent in this case, affects the survival model parameters. Models that provide such a description are known as secondary models and several models have been used extensively to calculate thermal and non-thermal processes. Some of them, taking a thermodynamics perspective, have used Arrhenius-like equations whereas others, on a more empirical bases, have used the Bigelow model as secondary models (Nunes, Swartzel, & Ollis, 1993). However, it is well known that microbial inactivation becomes more effective when the heating temperature reaches a certain threshold level, and beyond that temperature level, microbial inactivation rate increases significantly with temperature. The parameter *b*, which in an isothermal process is considered as the reciprocal of the microbial resistance to the treatment, under nonisothermal conditions is referred to as the rate of killing. To describe a relationship between the rate of killing and the processing temperature history having those characteristics, the log-logistic equation (Eq. 3) was selected (Campanella & Peleg, 2001):

$$b[T(t)] = Ln\ \{1 + \exp[k\ (T(t) - T_c)]\} \quad (3)$$

k and T_c are the corresponding survival parameters; T_c indicates the critical or threshold temperature from where significant inactivation starts, and k is the rate at which the inactivation rate rises after the temperature reaches that critical level (Peleg & Normand 2004). Eq. (3) is considered as a secondary kinetics model. The approach of solving equations (2) and (3) simultaneously is referred in this manuscript as the Peleg−log-logistic approach.

As discussed, another secondary model to describe the effect of the temperature on the inactivation rate has been using an Arrhenius-like equation (Eq. 4) which applied to the parameter $b\ (T)$ yields:

$$b(T) = A\, e^{\frac{-E_a}{RT}} \quad (4)$$

A is the frequency factor (also called the pre-exponential factor), E_a is the activation energy, R is the ideal gas constant, and T is the absolute temperature in Kelvin degrees (Jay, Loessner, & Golden, 2008). The Arrhenius equation is commonly used to describe the impact of the temperature on a chemical reaction rate, k. Thus, as an analogy, the reaction rate can be interpreted as the rate of killing or the rate of inactivation of microorganisms (André et al., 2019). The approach of solving equations (2) and (4) is referred in this manuscript as Peleg−Arrhenius approach.

The *ode45* function was combined with the nonlinear least-squares solver function, *lsqcurvefit* of the MATLAB ® Optimization Toolbox. This optimization function solves nonlinear curve-fitting problems to obtain the best model parameters that minimize the sum of squared error (SSE) between predicted and observed experimental data. During each iteration, the *ode45* function numerically solved the differential equation (Eq. 2), and *lsqcurvefit* adjusted the model parameters to minimize the difference between the survival data and the results of the numerical integration in that iteration. The iteration was repeated until the parameters that minimize SSE are achieved. The parameters k, T_c, and n for the Peleg−log-logistic approach, and the parameters A, E_a, and n for the Peleg−Arrhenius approach were obtained by this procedure.

The goodness of fitting of the two modeling approaches was evaluated by the mean square error (MSE) and the adjusted coefficient of determination (R^2-adj). A larger R^2-adj value (as it approaches 1) is indicative of good-fitting, whereas MSE measures the average of the squared deviation between the observed and the fitted values; a smaller MSE value (as it approaches 0) indicates a better fit of the model to the data.

4.3.10 Modeling temperature profile

Under nonisothermal conditions, a nonlinear empirical equation such as Eq. (5) can be used to describe these dynamic conditions. If this empirical equation is based on experimental data and no extrapolation is applied, it can be used as the experimental temperature profile. The apple juice temperatures recorded by the fiber-optic sensors in small time intervals (0.45s) are represented by a temperature vector $T = [T_0, T_1, T_2, \dots, T_f]$, which is input in MATLAB ® to describe temperature changes as a function of time. T_0 is the initial temperature of the juice whereas T_f is the final temperature reached in the process. The temperature at different time intervals, $T(t)_i$, was

calculated based on the previous temperature data point by Eq. (5). Constants α and β were estimated by nonlinear regression using the Solver tool from Microsoft Excel 2016™ and were inserted as constants in the MATLAB ® script. The coefficient of determination (R^2) and MSE was used to evaluate the goodness of fitting.

$$T(t)_i = T_{i-1} + \frac{t}{\alpha + \beta t} \tag{5}$$

An experimental heating curve was obtained for each target pathogen and power levels used in the study, and the values α and β determined were used for the integration of Eq. (3) and Eq. (4) to capture changes in the inactivation rate of the microorganisms for temperature conditions that are a function of time.

4.3.11 Model validation

The model parameters obtained from the Peleg−log-logistic and Peleg−Arrhenius approaches were used for predicting the inactivation curve of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium under microwave heating. For model validation and prediction capabilities independent experiments were carried out on commercially pasteurized apple juice inoculated with the target microorganisms and subjected to four power levels (480W, 600W, 720W and 1200W). All tests were run in duplicate at the specified treatment times to yield a total of 16 experimental survivor curves and models were utilized to predict the inactivation of both target pathogenic microorganisms under selected processing conditions. Pasteurized apple juice was purchased at a local supermarket (West Lafayette, Indiana) and stored at 4°C prior to treatment. The commercial juice has a solid soluble content of 11.5±0.03 °Brix, and a pH of 3.24 ±0.014, and after inoculation the samples were immediately subjected to microwave processing. The goodness of fit of the two modeling approaches was evaluated by the adjusted coefficient of determination (R^2-adj) and mean squared error (MSE).

4.4 Results and discussion

4.4.1 Modeling temperature profile of fresh apple juice during microwave processing

Time-temperature profiles of the apple juice samples during microwave heating were obtained by using the FISO Microwave Workstation™. The temperature histories followed similar patterns but with differences in the final temperature reached, which depended on the power level

applied. For instance, for the 720W power level, the final temperature reached was 90.4°C in 25s for fresh apple juices inoculated with *Escherichia coli*. However, when a 600W power level was applied for 25s, the final temperatures of the same sample reached 81.9°C. Typical real time-temperature histories for the fresh apple juice inoculated with *Escherichia coli* O157:H7 at the two microwave power levels (600 and 720W) are illustrated in Fig.8 along with the fitting of Eq. (5).

The parameters of Eq. (5) along with the R^2 and MSE are presented in Table 8, indicating a good agreement between data and the empirical model.

Table 8. Coefficients for the empirical heating regime (Eq. 5) fitted to the temperature profile for *E.coli* O157:H7 and *Salmonella* Typhimurium inoculated in fresh apple juice processed at the specified power levels:

		Fresh apple juice			
Pathogens	Power level (W)	α	β	R^2	MSE
E.coli O157:H7	720	3.28	0.72	0.9994	0.0728
	600	5.79	0.68	0.9995	0.0459
S. Typhimurium	720	3.95	0.65	0.9991	0.1350
	600	6.11	0.65	0.9994	0.0571

4.4.2 Inactivation of foodborne pathogens in apple juice by microwave pasteurization

Fig. 9 illustrates survival curves for *E. coli* O157:H7 and *Salmonella* Typhimurium in inoculated fresh apple juices during microwave treatment at the specified power levels of 600W and 720 W, which heated the samples to final temperatures of approximately 80°C and 90°C, respectively. Given the sample size and the heat penetration characteristics of the microwave radiation, it is reasonable to assume that temperatures in the sample were uniform. Inactivation data were determined every 5s starting at 5s until up to 25s. Statistical analyses suggested that the randomized complete block design employed was significant at the 95% confidence level for both used power levels. Microbial inactivation started after 10s of microwave heating for the two power levels used. Significant differences between treatment means were found after 10s until up to 25s for the 600W power level, but no significant differences were found between 20 and 25s when the 720W power level was used. Results indicated that the thermo-dependence of microwave lethality when lower power levels are applied is different between microorganisms possibly suggesting that

blocking in separate treatments, instead of using a mixed culture cocktail, was probably helpful in improving the precision to establish a comparison of treatment means. For more discussion of the used experimental design, refer to Montgomery (2013).

A 5-log reduction of *E. coli* O157:H7 and *S.* Typhimurium in fresh apple juices was accomplished when processing times were higher than 20s and 25s for 720 and 600W power levels, with final temperatures reaching up to 90 and 80°C, respectively. Thus, the selected processing parameters (power level, final temperature, treatment time) were able to meet the 5 Log_{10} reduction criteria requested by the U.S. FDA guidelines (USFDA, 2001). Another important characteristic of the survival curves obtained for all power levels and juices studied is the lack of linearity. Based on this characteristic, only non-linear models can be used to describe the effect of treatment time and temperature on the kinetic parameters.

4.4.3 Nonisothermal models used to describe survival curves

As illustrated in Fig. 9, *E.coli* O157:H7 and *Salmonella* Typhimurium survival data did not follow linear inactivation curves for any processing condition applied. For this reason, the Peleg non-linear model (Eq. 2) was used to describe the effect of treatment time and temperature on the kinetic parameters, and linear modeling was not employed. The microwave inactivation of the target microorganisms increased with treatment exposure time following a concave downward profile. As the exposure time progresses the microbial survivors are weakened, and gradually shorter times are necessary to destroy them. As a result, a characteristic downwardly concave survival curve is depicted (Peleg & Penchina, 2000). Related literature also showed downward survival curves due to microwave treatments (Goldblith & Wang, 1967; Papadopoulou et al., 1995; Song & Kang, 2016; Valero & Cejudo, 2014), which were similar to our findings. First-order kinetics are also described by the Weibull model when n=1 and, for these cases applying this model would result in under-processing of the juices and thus, a safety concern. The other reason of getting downward concave survival curves is that the temperature history is a function of time and as indicated by the secondary kinetics models (i.e. either the log-logistic or the Arrenhius equations used in this work – Eqs., 2 and 3) the lethal rate parameters increase with temperature and so does the microbial inactivation. The effects of temperature history are incorporated in Peleg's model given by Eq. 2.

By using the Runge-Kutta method (*ode45*) to solve the differential equation Eq. (2), and the *lsqcurvefit* function to find the optimal kinetics parameters that best fit the solution of this non-linear differential equation to the experimental data, the values of the kinetic parameters were obtained. They were k, T_c, and n for the Peleg−log-logistic approach and A, E_a, and n for the Peleg−Arrhenius approach. Table 9 presents the optimal parameters for both pathogenic microorganisms for the microwave treatment with power levels 600W and 720W and that yielded final target temperature of 80 and 90°C, respectively. As discussed, the goal was to build a modeling approach at power levels that would generate temperature rises equivalent to those obtained in conventional pasteurization methods, i.e. 80-90°C, in 25-30s. In this study, the target temperatures were achieved in 20-25s.

MSE and R^2-adj fell between 0.0078–0.2553, and 0.9230–0.9957, respectively for *E. coli* O157:H7, and between 0.0212–0.0755 and 0.9661–0.9902, respectively for *S.* Typhimurium, using the Peleg−log-logistic approach. For the Peleg−Arrhenius approach, these performance indices ranged from 0.0236 to 0.3467, and from 0.8865 to 0.9870, respectively for *E. coli* O157:H7; and 0.0260 to 0.1099 and 0.9502 to 0.9879, respectively for *S.* Typhimurium. Thus, the developed models were able to accurately predict the exposure treatment needed to achieve 5 Log_{10} reductions of the target pathogens in fresh apple juice by microwave pasteurization. Fig. 9 demonstrates that the models were highly satisfactory to describe inactivation for the two power levels examined. However, the statistics parameters MSE and R^2-adj indicate that the Peleg−log-logistic approach is slightly superior. The other aspect to be noted from the parameters obtained with the Peleg−Arrhenius approach is the inconsistency of the parameters E_a (Table 3, data for *S.* Typhimurium at power level of 600W). That inconsistency is due to the nature of the Arrhenius model. From Eq. (4), it can be seen that the parameter E_a can be calculated as the slope of a plot of $b(T)$ as a function of $1/T$, where T is the absolute temperature. The inversion of the temperatures, which in absolute values are high, compress the range the variation of that variable whereas on the other hand experimental error in the determination of the parameter b could increase with a result of a large variation in the slope and thus the value of E_a. Therefore, on this basis, it is demonstrated that the Arrhenius model is not a suitable secondary model to study microwave processing. Further evidence of the inadequacy of the Arrhenius model as compared with the log-logistic model is its low prediction capabilities which are discussed below.

Table 9. Optimized kinetic parameters after solving (Eq. 2 and Eq. 3) and (Eq. 2 and Eq.4) to describe survival data for *E.coli* O157:H7 and *Salmonella* Typhimurium inoculated in freshly prepared apple juice and treated by microwave heating at two specified power levels:

Pathogens	Power level (W)	Nonisothermal survival model (Eq.2)									
		Peleg−log-logistic approach					Peleg−Arrhenius approach				
		k(°C^{-1})	T_c (°C)	n	R^2-adj	MSE	A (s^{-1})	E_a(J.mol^{-1})	n	R^2-adj	MSE
E.coli O157:H7	720	0.1175	53.691	0.340	0.9230	0.2353	0.052	4178.096	2.009	0.8865	0.3467
	600	0.1168	53.275	0.405	0.9957	0.0078	0.010	3839.160	2.430	0.9870	0.0236
S. Typhimurium	720	0.0765	50.291	0.403	0.9661	0.0755	0.039	2350.123	1.850	0.9506	0.1099
	600	0.0881	50.948	0.473	0.9902	0.0212	0.005	596.876	2.314	0.9879	0.0260

4.4.4 Model validation

To test the accuracy and the prediction capability of the kinetic parameters obtained from the two modeling approaches reported in Table 9, a new set of experiments was performed using commercial apple juice samples inoculated with the target microorganisms and subjected to four power levels of microwave heating (480, 600, 720, and 1200W). Data of survival curves are represented as means and standard errors. The empirical temperature profiles obtained with those power levels, along with the fitted non-linear heating regime (Eq. 5), are presented in Fig. 10. The survival parameters obtained from the two modeling approaches, the Peleg−log-logistic approach (Eq. 2 and 3) and the Peleg−Arrhenius approach (Eq. 2 and 4) reported in Table 9, were used to predict the inactivation curve of *E.coli* O157:H7 and *Salmonella* Typhimurium in commercial apple juice inoculated with the target microorganisms and treated at other microwave conditions (power levels 480, 600, 720, and 1200W). The statistical performance indices of the validation analysis are listed in Table 10.

Fig. 11 shows predictions for *E.coli* O157:H7 survival using parameters obtained from the Peleg−log-logistic approach (Fig. 11A) and parameters obtained from the Peleg−Arrhenius approach (Fig. 11B). Moreover, Fig. 11C illustrates predictions of *Salmonella* Typhimurium using paramaters obtained from the Peleg−log-logistic approach and Fig. 11D predictions with parameters obtained from the Peleg−Arrhenius approach. It must be noted that all these predictions on inoculated commercial apple juice samples were obtained using survival parameters obtained from inoculated fresh apple juice samples processed at the 600W power level, i.e. they can be considered true predictions.

Fig. 12 shows inactivation prediction of the target pathogens using survival parameters obtained from treatements using the 720W power level and tested on freshly prepared apple juice (see Table 9). The predicted survival curves were quite similar, with a slightly better prediction using the 600W power level data. In Figs. 11 and 12 is clear that the Peleg-log-logitistic approach has a superior prediction capacity. The advantage of using this approach is that the rate of killing, as expressed by Eq. (3), provides a more realistic description of the inactivation process which is based in the critical temperature parameter T_c beyond which inactivation is enhanced. According to this model (Eq. 3) the inactivation rate $b\ (T)\sim 0$ at $T \leq T_c$, and at $T \geq T_c$ $b\ (T)\sim k\ (T(t) - T_c)$, i.e. the inactivation rate increases sharply at temperatures higher than the critical or threshold temperature (Peleg et al., 2002; Peleg & Normand, 2004). The assumption that the inactivation

rate is approximately zero below the critical temperature is adequate, as the log reductions for times between 0 to 10s were not significantly different according to a Tukey's analysis (difference between the means were less than the Tukey's minimum significant difference). Thus, possibly indicating that the inactivation rate was approximately zero whitin that period range. The magnitude of T_c obtained for *S.* Typhimurium was within 50-51ºC, and 53-54ºC for *E.coli* O157:H7. The threshold range for *E.coli* is in accordance with Siguemoto et al., (2018) who reported that for conventional thermal processing no inactivation of *E.coli* O157:H7 was detected at temperatures below 55ºC. The slightly lower value of the critical temperature for *Salmonella* is in agreement with its observed higher log reduction level of 0.497 to 2.480 when exposed to 600W from 10s to 15s, whereas for the *E.coli* O157:H7 population the log reduction changed from 0.340 to 1.687 under the same conditions (see Fig. 9); thus, suggesting that the inactivation of *Salmonella* started at lower temperature when compared to *E.coli* O157:H7. Similarly, Song and Kang (2016) observed that *Salmonella* Typhimurium showed less resistance than *E.coli* O157:H7 to microwave heating. The observed differences in the microorganism's heat sensitivity are of no concern for the purposes of this investigation. However, the threshold range of 50-54ºC necessary to promote microbial inactivation should assist juice processors in determining the target temperature required to enhance the inactivation level the of these pathogens.

The most important criterion to test the suitability of a modeling approach to describe a food process is to asses if it can be used to predict survival of similar microorganisms in a similar or related medium, but treated at different processing conditions. In that sense, the performed validation analysis demonstrated that the experimental data obtained for all power levels can be, almost invariably, appropriately described by parameters obtained from the Peleg−log-logistic approach, regardless if the survival parameters were obtained at 600 or 720W power levels (Figs. 11 and 12). However, the Peleg-Arrhenius approach did not have good prectiction ability. In other words, predicting the inactivation kinetics of the target pathogens using the Peleg−Arrhenius approach did not result in a substantial agreement between the experimental data points and the predicted survival curves, independently if the parameters were obtained at 600 or 720W power levels (Figs. 11B, D and Figs. 12B, D). As discussed, originally the Arrhenius equation was developed to describe succesfully the effects of temperature in chemical reactions. However, results of this study show that a simple analogy between chemical reaction rate and microbial inactivation rate is not always applicable. As can be seen in Figs. 11B, D, and Figs. 12B, D, when

the pathogens were exposed to different power levels, the Peleg−Arrhenius model did not capture the pertinent aspects of the inactivation as a function of the temperature history correctly. One can argue that it may have somewhat worked to describe survival curves of inoculated fresh apple juice samples treated at the two power levels, from which the survival parameters were obatined (Fig. 9), but that was a simple a fitting procedure and not prediction. However, Figs. 11 and 12 are clearly showing that the approach would not be a recommendable one to predict survival curves for other microwave processing conditions and other materials.

Despite the numerous and even recent reports about the use and suitability of the Arrhenius equation to describe inactivation curves (André et al., 2019; Dahlquist-Willard et al., 2016; Lee, Heinz, & Knorr, 2011; Murphy, Marks, Johnson, & Johnson, 2000; Fachin, Van Loey, Indrawati, Ludikhuyze, & Hendrickx, 2002), it appears that its validity has actually been taken for granted (Peleg & Corradini, 2011). Originally the Arrhenius equation was utilized to model simple collisions of gas molecules that would react when they acquire enough kinetic energies to overcome electrons repulsion. Similarly, the equation could also describe the disintegration of molecules (Peleg & Corradini, 2011; Vallance, 2017). However, as stated by Peleg, Normand and Corradini (2012) and also experimentally and theoretically demonstrated in this work, using the Arrhenius equation to predict microbial inactivation does not see to be appropiate. Furthermore, including the gas constant (R= 8.314 J $mol^{-1}K^{-1}$) in calculations involving microorganisms may be confusing. In a potential energy pathway, for an exothermic reaction, the difference between the transition state and the reactants is called the energy of activation with units of joule per mole (Vallance, 2017). In light of this concept, the quantities of the reactants are expressed in “moles”. But using the Arrhenius reaction rate as an analogy to describe inactivation rate, and then calculating the energy of activation in joule per mole of bacteria, leads to a simple but challenging question like for example, what is the mass of a a mole of bacterial cells? By using some simple assumptions Peleg et al. (2012) estimated that 1 mol of bacteria may have a mass of $6x10^5$ metric tons. Moreover, literature using the Arrhenius equation for kinetic studies would assume that the inactivation process caused by heat has a fixed energy of activation, or that the inactivation effects of heat is qualitatively the same at low and high temperatures (Peleg et al., 2005). Such assumptions are not appropriate, considering that inactivation effects are enhanced when the temperature is increased (see for example data of *E. coli* and *S.* Typhimurium in Figs. 11 and 12) and even at temperatures below T_c inactivation is not statistically significant. For these reasons,

the application of the Arrhenius equation to systems that are different than the ones for what the equation was derived must be seriously challenged (Peleg et al., 2012). The findings of this manuscript clearly show its lesser adequacy in terms of prediction ability. The comparison of the two modeling approaches suggests that the higher quality of the Peleg−log-logistic approach to predict microbial survival at other microwave processing conditions would be the preferred one for modeling and prediction purposes.

Literature has shown that there is interest in studying microbial inactivation kinetics under microwave heating for a variety of foods and microorganisms. Although the relevant and reviewed literature in microwave inactivation kinetics presents their worthiness (Chapter 2), none of the studies actually explored the validation potential as it is explored in the present study. Therefore, this investigation is among the first reports on the development and validation of a mathematical modeling approach to predict the inactivation of pathogenic microorganisms in apple juice subjected to microwave heating. To the best of our knowledge, no previous studies have used the Peleg nonisothermal differential equation in combination with a log-logistic equation and the temperature history. Neither a comparison of the prediction ability of this approach with other that employs the widely used Arrhenius equation has been reported to model and predict inactivation of *E. coli* O157:H7 and *Salmonella* Typhimurium in apple juices treated by microwave. The developed model was proven to be reasonably accurate for predicting the inactivation of *E. coli* O157:H7 and *Salmonella* Typhimurium in fresh apple juices using microwave heating for pasteurization at 80-90°C for 20-25s. Therefore, the developed model may be further used to facilitate parameter selections to scale up or optimize microwave heating as a pasteurization intervention for apple juice.

Regarding microwave effects, the debate whether there are non-thermal inactivation effects due to the microwave process is a subject of constant discussion in the scientific community. As reviewed by Chandrasekaran et al. (2013), there is no proof of lethal effects of microwave radiation in the absence of temperature or other stress conditions. In fact, a study conducted by the U.S. Department of Agriculture at Wyndmoor (PA), developed a system to distinguish thermal and non-thermal effects of microwave energy. The study was conducted to inactivate *E.coli* K-12, *Listeria monocytogenes*, *Enterobacter aerogenes*, *Pediococcus sp*. and yeast, in fluids such as liquid egg, beer, water, apple juice, apple cider, and tomato juice. The researchers found that there is no evidence that microwave energy can inactivate microorganisms without thermal energy or other

stresses such as pH (Kozempel, Cook, Scullen, & Annous 1999). From a chemical reaction perspective, Kappe, Pieber, and Dallinger (2013) argued that the energy of the microwave photon is far too low to induce chemical reactions and that the observed effects in organic reactions are purely a result of bulk thermal phenomena. Ultimately, the U.S. FDA (2000) resolved that considering non-thermal inactivation effects is inadequate to explain microbial inactivation under microwave heating. In modeling inactivation kinetics of microwave heating, it is therefore recommended to include only thermal effects in the modeling (Chandrasekaran et al., 2013; US FDA, 2000).

Table 10. Statistical performance indices of the validation and prediction analysis.

Pathogens	Model built in the power level (W)	Power level (W) for validation	Nonisothermal survival model (Eq.2)			
			Peleg−log-logistic approach		Peleg−Arrhenius approach	
			R^2-adj	MSE	R^2-adj	MSE
E. coli O157:H7	600W	1200	0.9158	0.2497	*	27.5752
		720	0.8582	0.4604	0.6528	1.1272
		600	0.9815	0.0424	0.9705	0.0676
		480	0.9550	0.0152	*	3.6483
	720W	1200	0.9431	0.1688	*	18.5556
		720	0.8033	0.6387	0.8375	0.5275
		600	0.9057	0.2158	0.9002	0.2286
		480	0.7714	0.0774	*	6.7117
S. Typhimurium	600W	1200	0.9612	0.1675	*	9.6296
		720	0.9360	0.1585	0.9572	0.1059
		600	0.9118	0.2187	0.9793	0.0514
		480	*	0.5527	*	6.4328
	720W	1200	0.7849	0.9285	*	7.1954
		720	0.9454	0.1353	0.9735	0.0656
		600	0.7181	0.6990	0.9303	0.1728
		480	0.0560	0.3419	*	7.1490

*Negative values were obtained indicating that the model could contain terms that do not help to predict the response.

4.5 Conclusions

Microwave processing occurs under nonisothermal conditions. The present work developed a reliable model to describe inactivation kinetics of *E. coli* O157:H7 and *Salmonella* Typhimurium under those conditions by incorporating into the process calculation the temperature history. All experimental survival curves lacked linearity, thus a first-order kinetics model was not adequate and not employed. Survival curves were modeled through the solution of the Peleg non-linear differential equation. The kinetic parameters were obtained by a numerical approach using MATLAB ®. Fresh apple juice was pasteurized by temperatures reaching up 80-90ºC in 25s with log pathogen reduction achieving 7-log units, thus meeting the FDA guidelines for processed juices. The modeling approach developed in the present work was further validated using other processing conditions and other samples. The validation analysis of the model showed that there was reasonably agreement between experimental and predicted data, but more specifically, the Peleg−log-logistic approach showed better prediction capability. It also showed a means to determine the critical temperature level to enhance microbial inactivation under microwave heating. Results clearly showed that, although the two approaches were able to appropriately describe the survival curves under nonisothermal treatment, the use of the Arrhenius approach did not have any prediction capabilities (Figs. 11B, D and 12B, D). Although the Arrhenius equation has been largely used to asses the effect of temperature on the rate constants of chemical reactions, its application to food processing has been seriously challenged (Peleg & Corradini, 2011; Peleg et al., 2002, 2012), and its less prediction capability is clearly demonstrated in this article.

U.S. FDA specifies that it is the juice processor's responsibility to validate the food process in order to assure its safety effectiveness by consistently achieving a 5-log or greater reduction. Thus, it is expected that the modeling approach presented and its validation in this work should assist the food industry in accomplishing this mission.

Author's contribution

Gabriella Mendes C. de Oliveira conceived the idea of this study, the experimental design, conducted the experiments, interpreted the data, wrote the code, ran the model, analyzed the output data and wrote the manuscript. Osvaldo H. Campanella supervised the modeling analysis, Amanda J. Deering supervised the microbiological experiments, and M. Fernanda San Martin-Gonzalez

assisted with microwave pasteurization tests. All contributed to the final version of the manuscript. The authors declare that there is no conflict of interest.

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4.6 Figures

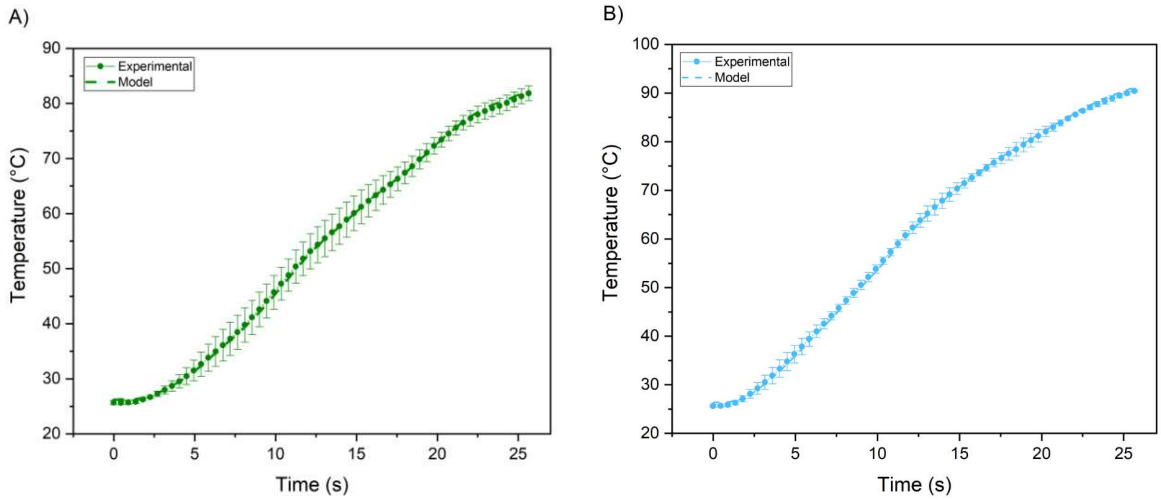

Fig. 8. Experimental temperature profile of fresh apple juice inoculated with *E. coli* O157:H7 for (A) 50% power: 600W, (B) 60% power: 720W, and fit of experimental values by Eq. (5).

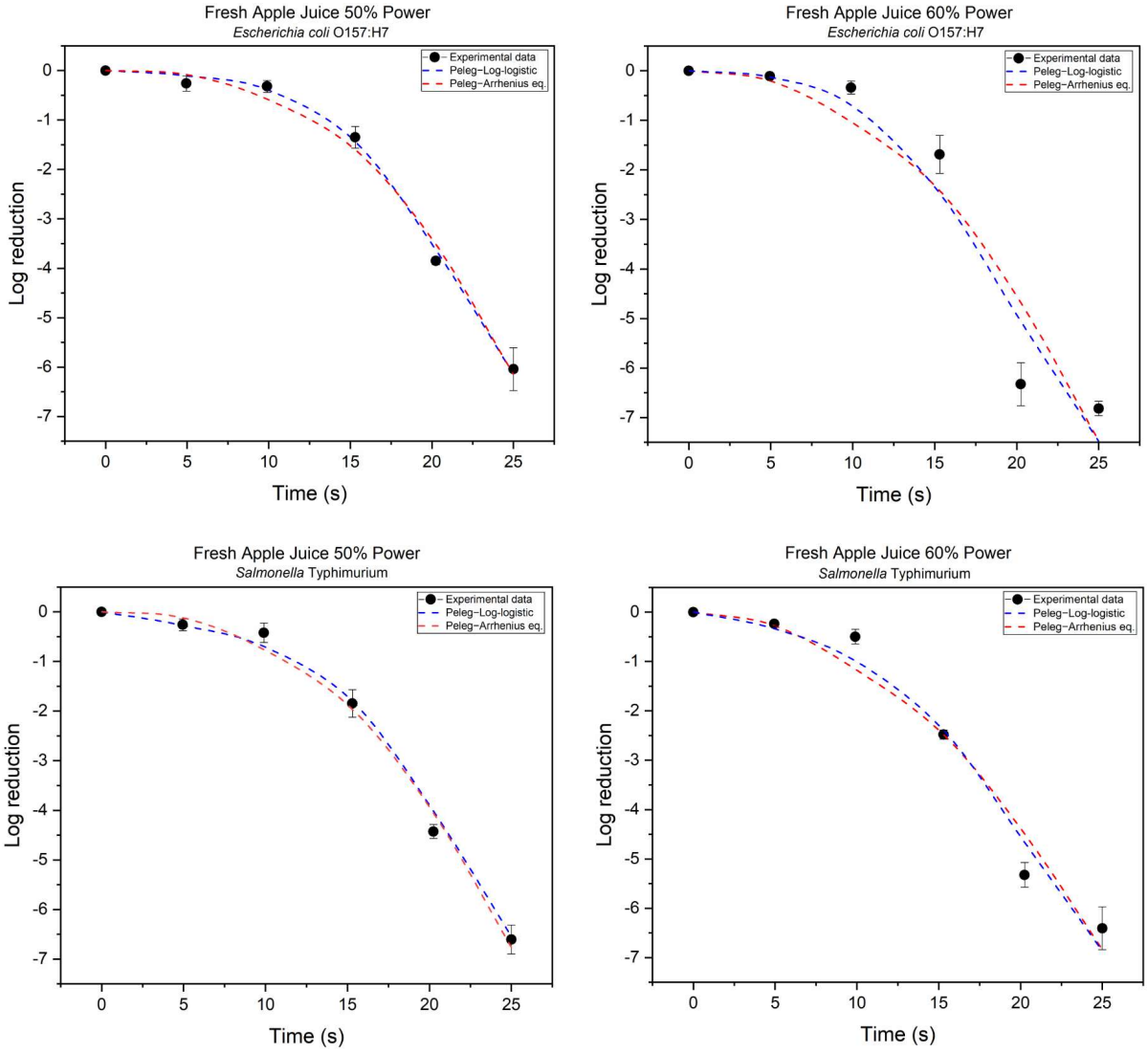

Fig. 9. Survival curves of *E. coli* O157:H7 and *Salmonella* Typhimurium in fresh apple juice during microwave heating. Dashed blue line represent the Peleg−log-logistic approach and the red line represents the Peleg−Arrhenius approach.

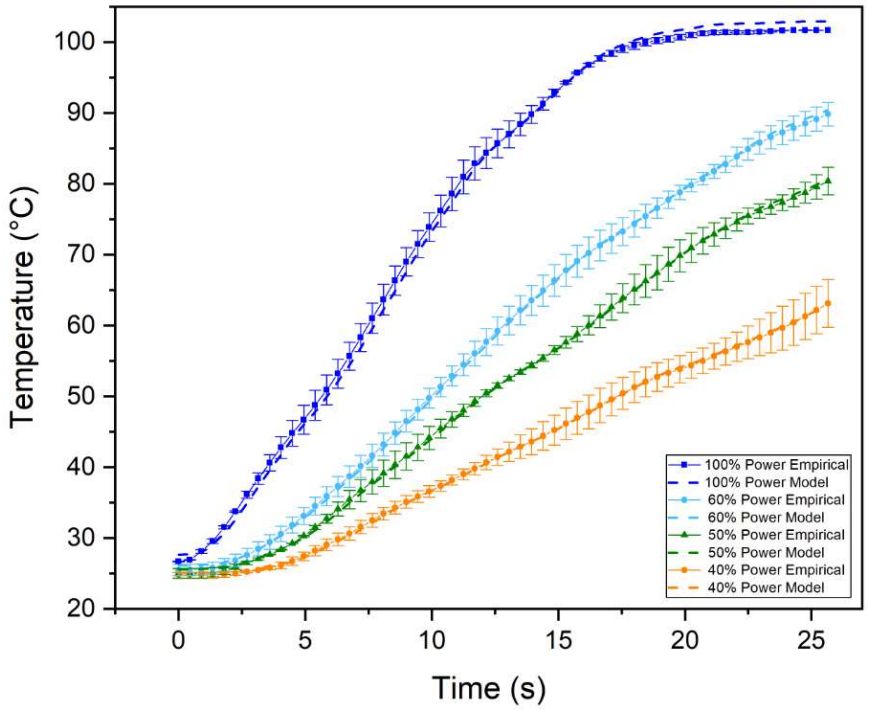

Fig. 10. Temperature profile of inoculated commercial apple juice used for validation purposes treated with four microwave power levels and modeled by Eq. (5). Dots represent data and dashed lines the model: dark blue color illustrates:1200 W; light blue color illustrates: 720W; green color illustrates: 600W; orange color illustrates: 480 W.

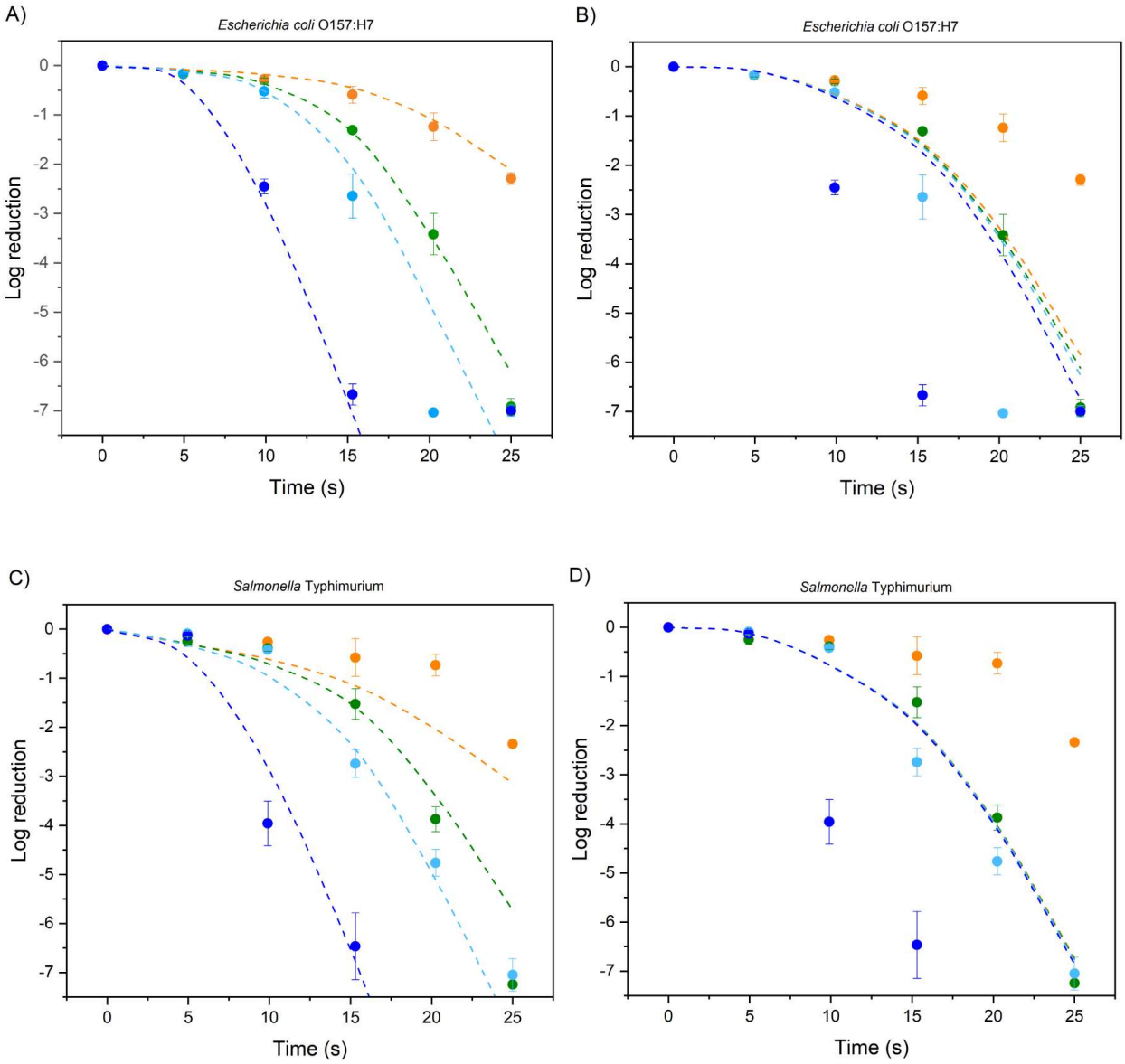

Fig. 11. Prediction and validation analysis at other power levels and using commercial apple juice samples. Symbols are experimental points and lines are model prediction. 1200W (dashed dark blue symbol and line); 720W (dashed light blue symbol and line; 600W (dashed green symbol and line), and 480 W (dashed symbol and orange line). (A) Peleg−log-logistic approach and (B) Peleg−Arrhenius approaches for *Escherichia coli* O157:H7; (C) Peleg−log-logistic and (D) Peleg−Arrhenius approaches for *Salmonella* Typhimurium in commercial apple juice. Prediction parameters were obtained using a treatment with a 600W microwave power level on fresh apple juice samples.

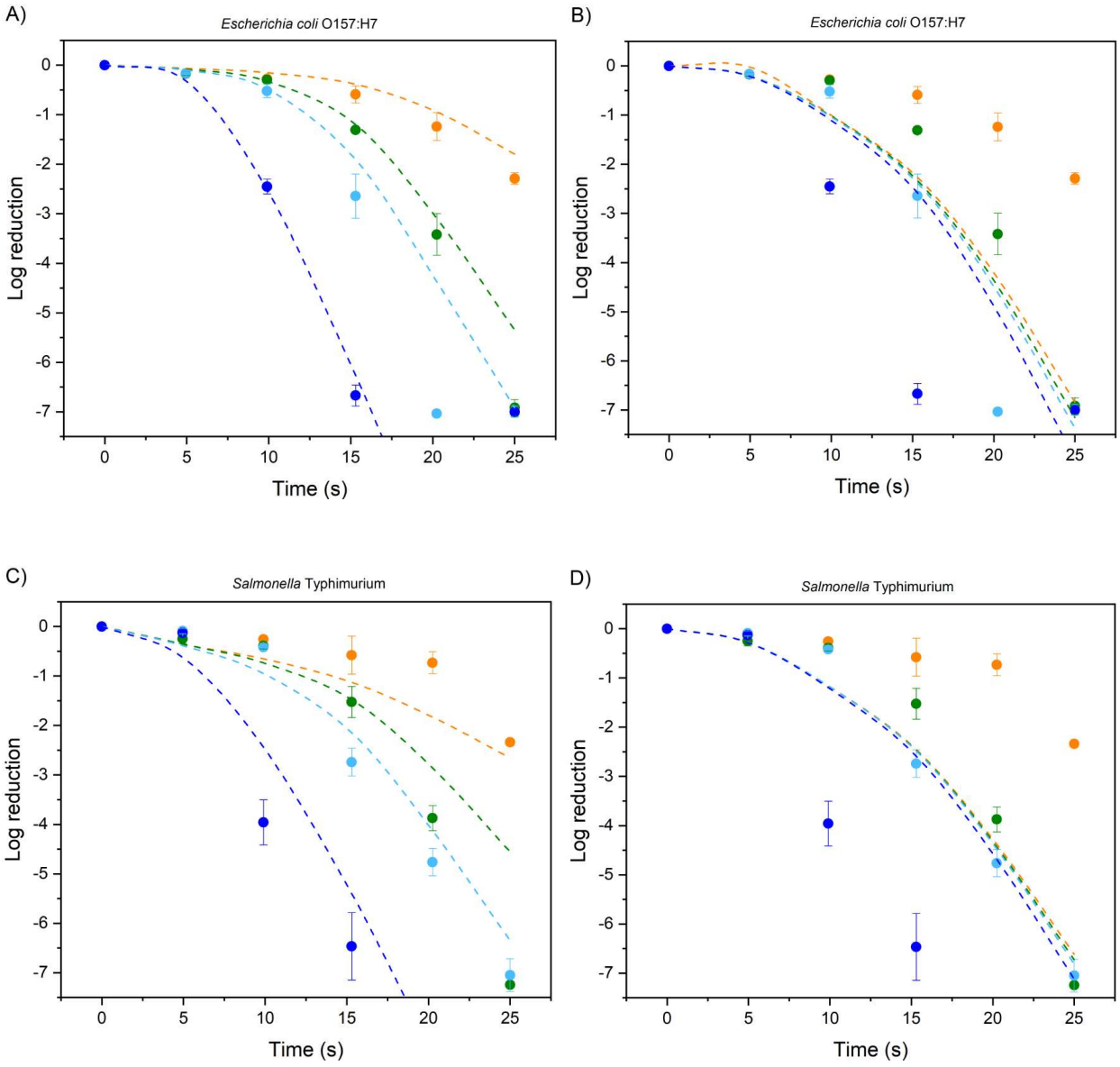

Fig. 12. Prediction and validation analysis at other power levels. Symbols represent experimental points whereas lines are predictions. 1200W (dashed dark blue symbol and line); 720W (dashed light blue symbol and line); 600W (dashed green symbol and line), and 480 W (dashed orange and line): (A) Peleg−log-logistic and (B) Peleg−Arrhenius approaches for *Escherichia coli* O157:H7; (C) Peleg−log-logistic and (D) Peleg−Arrhenius approaches for *Salmonella* Typhimurium in commercial apple juice. Prediction parameters were obtained using a treatment with a 720W microwave power level on fresh apple juice samples.

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CHAPTER 5. MODELING INACTIVATION OF *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* TYPHIMURIUM IN JUICES BY PULSED ELECTRIC FIELDS; THE ROLE OF THE ENERGY DENSITY

5.1 Abstract

Electric field intensity, pulse repetition rate, treatment time, and food electrical conductivity are the main parameters for microbial inactivation by PEF processing. The objective of this study was to integrate these parameters into one factor, energy density, and correlate the inactivation rate of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium with this integral PEF factor. A continuous bench scale PEF system (OSU-4H) treated the inoculated apple cider samples. Non-linear survival curves were modelled by numerically solving a differential equation using the Runge-Kutta method (*ode45* in MATLAB ®). The *lsqcurvefit* function in MATLAB ® estimated the kinetic parameters. Samples were treated under four combinations of electric field strength (20, 25, 30 and 35 kV/cm), and five pulse repetition rates per second (500, 750, 1000, 1250, 1500 pps). The developed model successfully described the survival of these pathogens. The validation with different juices and PEF treatment conditions demonstrates that the model satisfactorily predicted the experimental data as well as the data from literature. This suggests that the model is suitable for simply and precisely estimating the inactivation rate of foodborne pathogens in juices by PEF processing.

Key words: pulsed electric fields, *E. coli* O157:H7, *Salmonella*, modeling, validation, energy density

5.2 Introduction

Pulsed electric field (PEF) is a promising nonthermal alternative to food processing (Altunakar & Barbosa-Cánovas, 2011). In the PEF process, the food is treated between two electrodes and exposed to a high intensity electric field (typically 20-80 kV/cm) (Barbosa-Cánovas, Pierson, Zwang, & Schaffner, 2000) for a short period of time from microseconds to milliseconds (Barba et al., 2015; Mohamed & Eissa, 2012). Literature has shown the capacity of the PEF treatment to inactivate a range of microorganism present in several food products (Evrendilek,

Zhang, & Richter, 1999; Evrendilek et al., 2000; Gurtler, Bailey, Geveke, & Zhang, 2011; Gurtler, Rivera, Zhang, & Geveke, 2010; Liang, Cheng, & Mittal, 2006; Liang, Mittal, & Griffiths, 2002; Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2008a, 2008b, 2008c; Niu et al., 2019; Wei, Yang, Shen, Zhang, & Chen, 2013) while preserving nutritional and quality attributes (Caminiti et al., 2011; Elez-Martínez & Martín-Belloso, 2007; Guo et al., 2014; Jin, Guo, & Yang, 2014; Jin, Yu, & Gurtler, 2017; Kantar et al., 2018; Turk, Vorobiev, & Baron, 2012). The application of PEF has been discussed in several reviews (Barba et al., 2015; Buckow, Ng, & Toepfl, 2013; Li & Farid, 2016; Vega-Mercado et al., 1997), and many of them have focused in the design of the treatment chamber and main processing parameters (Huang & Wang, 2009; Toepfl, Siemer, Saldaña-Navarro, & Heinz, 2014).

PEF process parameters affecting microbial inactivation include electric field intensity, shape and width of the pulse, pulse frequency and treatment time (Altunakar & Barbosa-Cánovas, 2011; Martín-Belloso & Soliva-Fortuny, 2011). Among them, the electric field intensity and the number of pulses applied (treatment time) are considered key parameters (Altunakar & Barbosa-Cánovas, 2011) of the process.

Inactivation of microorganisms increases with applying higher electric field strengths (Amiali & Ngadi, 2012; Martín-Belloso & Soliva-Fortuny, 2011; Zhang, Barbosa-Cánovas, & Swanson, 1995). The critical electric field intensity (E_c) is a threshold parameter below which no significant effects on microbial viability occur (Schoenbach, Joshi, & Stark, 2000) or conversely, above which the microbial inactivation rate increases with the applied electric field (Zhang et al., 1995). Effects of electric fields on microbial inactivation can be explained by a mechanism of pore formation in the cell membrane (Schoenbach et al., 2000). When an electric field is applied, charges accumulate at the cell membrane generating a transmembrane potential that increases with the electric field. The increase in the transmembrane potential leads to structural changes in the membranes that culminate in the formation of pores and, ultimately, irreversible breakdown occurs leading to a mechanical destruction of the membrane and subsequent cell death (Buckow et al., 2013; Pagán & Mañas, 2006; Schoenbach et al., 2000).

Treatment time plays a significant role in inactivation as its increase generally results in increased microbial inactivation (Mosqueda-Melgar et al., 2008b; Toepfl et al., 2014). The treatment time can be calculated by multiplying the pulse repetition rate, the pulse width, the residence time of the product in the chamber, and the number of PEF treatment chambers (Jin,

2017). However, the chamber geometry may influence the electric field remaining above the critical electric field, this happens for instance, with co-linear configurations for which the field intensity distribution must be taken into account when calculating the residence time. Consequently, the energy density is a parameter that has been suggested as a PEF control/intensity factor (Heinz, Álvarez, Angersbach, & Knorr, 2002; Toepfl et al., 2014).

The energy density accounts for the influence of the electric field intensity, the pulse repetition rate and its width, along with the treatment and residence times, in addition to the electrical properties of the treated food such as the electrical conductivity (Jin, 2017). The aim of this study was to correlate the kinetics of microbial inactivation with this integrated PEF parameter in order to facilitate the selection of adequate PEF processing parameters in the production of safe food products.

Escherichia coli O157:H7 and *Salmonella* Typhimurium have been involved in several food poisoning outbreaks in a variety of food products including juices and ciders (Vojdani, Beuchat, & Tauxe, 2008). In response to these juice-associated outbreaks, the FDA published the Hazard Analysis and Critical Control Point (HACCP) regulation that requires that juice processors apply a treatment that achieve at least a 5-log reduction of pathogens of public health concern (USFDA, 2001). Based upon foodborne illness outbreak data, *Salmonella* is accepted as one of the pertinent pathogen in citrus juices and *Escherichia coli* O157:H7 in apple juice (Danyluk, Goodrich-Schneider, Schneider, Harris, & Worobo, 2012).

The design of effective PEF treatments to achieve the safety level required by the HACCP regulation can be facilitated if mathematical models to predict microbial inactivation are developed. Therefore, this research is proposing a modeling approach to predict microbial inactivation under more realistic PEF conditions.

For the development of the model, it is hypothesized that the microbial population inactivation rate is a function of the energy density, and when the external electric field reaches a critical threshold, microbial inactivation starts. To test this hypothesis, the specific objectives were: (1) to investigate the effects of the energy density parameter in the PEF treatment on the inactivation of the target microorganisms *Escherichia coli* O157:H7 and *Salmonella* Typhimurium; (2) to develop a modeling approach that incorporates the energy density to estimate inactivation of the targeted pathogens; (3) to estimate the corresponding survival parameters; (4) to validate the modeling approach and the model survival parameters in other commercially operations

involving pasteurized apple cider, as well as published data for other juices and other PEF conditions, and ultimately (5) to test the modeling approach's capability in estimating survival parameters using a PEF system having a different number of treatment chambers set at other pulse repetition rates, pulse width, residence time, and electric field strengths.

5.3 Materials and Methods

5.3.1 Apple Cider

Apple cider (Zeigler's ®, Landsdale, PA) was purchased at a local retail grocery store (Wyndmoor, PA, USA) stored at 4°C and used for the model development. Apple cider for validation analysis was provided by a cooperating industry partner, wishing to remain anonymous. This apple cider was also stored at 4°C.

Soluble solid content (°Brix) for the samples was measured using a digital laboratory refractometer (AR200 Reichert, Reichert Inc., Depew, NY, USA). The pH was measured using a benchtop pH-meter (TS625 Thomas Scientific, Thermo Electron Corp., Beverly, MA, USA) previously calibrated with standard buffer solutions. The electrical conductivity was measured using a conductivity meter (CON6, Oakton Instruments, Vernon Hills, IL, USA).

5.3.2 Pulsed electric field system

A continuous flow bench-scale PEF processing system (OSU-4H Model) located at the Eastern Regional Research Center (ERRC), Agricultural Research Service, USDA (Wyndmoor, PA), which can discharge bipolar square-waves pulses was used to process the apple cider samples. The PEF system is composed of six co-field treatment chambers in series, each containing two stainless steel electrodes with diameter of 0.23cm and a gap distance of 0.29cm. After passing through each pair of treatment chambers, the juice sample was cooled by passing it through a cooling coiled stainless steel tube submerged in a water bath set at 5°C (Polystat®, Cole-Parmer, Vernon Hills, IL, USA) in order to avoid temperature rises during the PEF treatment. Type K thermocouples attached to a multiple input digital thermometer (Omega HH374, Omega Engineering, Inc., Norwalk, CT, USA) were used to monitor the sample inlet and outlet temperatures. The pulse waveform, voltage, and current were monitored using an oscilloscope (TDS-210, Tektronix, Beaverton, OR, USA) connected to the PEF apparatus. The high voltage pulse generator (Model 9410, Quantum Composers, Inc., Bozeman, MT, USA) provided bipolar

square-wave pulses with the pulse repetition rate (frequency) 500 – 2000 pulses per second (pps) and a pulse width (pulse duration) of 2μs. The sample flow rate, set at 60ml/min, was controlled by a digital gear pump (Digital pump 75211-30, Cole-Parmer, Vernon Hills, IL, USA) previously calibrated following pump manufacturer specifications. Before and after each treatment the PEF fluid handling system was cleaned with a 10% household bleach solution and rinsed with sterile deionized water. Prior to treatment, autoclaved juice samples were circulated through the PEF system for 10 min, followed by pumping the inoculated apple cider to be treated. After treatment, PEF fluid handling system was disinfected by pre-rinsing the system with high flow-rate deionized water, followed by a recirculation with a 10% household bleach solution for 1 hour, and a final rinse with sterile distilled water for 30min. The absence of microorganisms in the wash water after disinfection confirmed no cross contamination of apple cider samples.

5.3.3 Pulsed electric field processing

Apple cider samples (12.5°Brix, pH 3.45, conductivity of 2088μS/cm) were treated with electric field strengths of 20, 25, 30, 35kV/cm at repetition rates of 500, 750, 1000, 1250 and 1500pps. Treatment combinations of these process parameters were selected to reduce bacterial populations from 1 to 6 log CFU/ml. All the variables combinations, except 1250 and 1500pps at 35kV/cm, were tested. Repetition rates of 1250 and 1500pps at 35kV/cm were not tested because the target 5 log reduction was achieved at 30kV/cm, thus it was not necessary to increase the field strength beyond that point. All the experiments combinations were performed in triplicates and repeated three times in different days.

5.3.4 Microbial growth conditions and inoculation

Escherichia coli O157:H7 (ATCC 43894) and *Salmonella* Typhimurium (ATCC 14028) were from ERRC culture collection and were stored on Tryptic Soy Agar slants (TSA, Difco, Becton Dickinson, Sparks, MD) in borosilicate screw-cap test tubes at 4°C. A loop full of culture was inoculated into 10ml of Tryptic Soy Broth (TSB, EMD, Merck KGaA, Darmstadt, Germany) and incubated at 37°C for 18h with continuous agitation (110 rpm) prior to experiments. Bacterial cultures were harvested by centrifugation at 4000rpm at 4°C for 10 min, the resulting pellets were washed in sterile 0.1% peptone water (BD/Difco Laboratories, Sparks, MD, USA) and centrifuged again. The supernatant removed, and the resulting pellets were resuspended in 10ml of 0.1%

peptone water. The initial cell concentration of each bacterial culture was approximately 10^9 CFU/ml. Finally, cells were inoculated in apple cider at a 1:50 ratio to obtain initial populations of 7 log CFU/ml and immediately PEF treated.

5.3.5 Enumeration of survivors

After PEF treatments, sample volumes of 1ml were 10-fold serially diluted in 9ml of sterile 0.1% peptone water, and 0.1ml of the diluted sample was spread-plated in duplicate onto selective media Sorbitol MacConkey Agar (SMAC) for *E. coli* O157:H7, and Xylose Lysine Tergitol 4 Agar (XLT4) for *S.* Typhimurium. All dilutions were plated in duplicate and plates were incubated at 37°C for 24h before enumeration.

5.3.6 Experimental design and statistical analysis

Experiments were carried out as a randomized block factorial design to evaluate the effects of pulse repetition rate (500, 750, 1000, 1250, 1500pps) and electric field strength (20, 25, 30kV/cm) in the population reductions of *E. coli* O157:H7 and *S.* Typhimurium specified as blocks. Mean and standard errors were calculated for each treatment. Inactivation data were analyzed using the Statistical Analysis System software (SAS 9.4, SAS Institute Inc, Cary, NC, USA). The GLIMMIX procedure of SAS was used to evaluate pulse repetition rate, field strength, and microorganism as fixed effects. Tukey's test was used to determine whether treatment combinations were significantly different (p<0.05).

5.3.7 Energy density input

The energy density (J/ml) delivered to the apple cider sample, as above discussed an integrated parameter during PEF processing, was calculated by Eq. (1) (Jin, 2017):

$$\Delta E = E^2 * f * \tau * \sigma * R_t * nc \quad (1)$$

where E is the electric field strength (kV/cm), f is the repetition rate (pulses per second), τ is the pulse width (μs), σ is the electrical conductivity of the sample (S/cm), R_t is the residence time (s) calculated by Eq. (2), and nc is the number of PEF chambers, whereas v is the volume of the chamber (cm^3), q is the flow rate in ml/s.

$$R_t = \frac{v}{q} \quad (2)$$

5.3.8 Modeling of survival curves

A mathematical model based on the Peleg and Penchina (2000) approach was used to model the survival curves of *E. coli* O157:H7 and *S.* Typhimurium at different energy densities. The Peleg and Penchina (2000) equation was originally developed to calculate the survival ratio of a given microorganism to nonisothermal heat treatments. The applicability of this equation can be extended to PEF treatments in the present study and presented as follow:

$$\frac{\mathrm{d}\log S(\chi)}{d\chi} = -b(\chi)\, n(\chi) \left(\frac{-log\, S(\chi)}{b(\chi)}\right)^{\frac{n(\chi)-1}{n(\chi)}} \quad (3)$$

where $S(\chi)$ is the survival fraction; χ is the specific energy ΔE or energy density in J/ml. The logarithm of survival ratio $S(\chi) = (N_f/N_0)$ can be plotted against a variety of energy densities calculated for each repetition rate applied at different electric field strengths. N_f is the final number of survivors after treatment (CFU/ml) and N_0 is the initial number of the microbial population (CFU/ml).

This non-linear differential equation was solved numerically as an initial value problem (the initial condition specified at $\log S(0) = 10^{-7}$ to avoid numerical problems associated to the singularity of the log function at the zero value) using the Runge-Kutta method. The mathematical software MATLAB ® (Mathworks®, Natick, MA, USA) and its solver *ode45* was used to solve Eq. (3) numerically.

In thermal processing, microbial inactivation is more effective as the temperature reaches a lethal level. Beyond this 'transition' or critical level, inactivation increases linearly with temperature (Peleg & Normand, 2004). The same concept can be applied to PEF processing but, instead of temperature, the energy density applied to the system (Eq.1) can be assumed as the control parameter that defines that critical level. The energy density dependence pattern of microbial inactivation can be described the log−logistic equation (Campanella & Peleg, 2001):

$$b(\Delta E) = Ln\,\{1 + \exp[k\,(\Delta E - \Delta E_c)]\} \quad (4)$$

k and ΔE_c are characteristic survival parameters and as discussed ΔE_c indicates the critical or threshold energy density after which significant inactivation starts, whereas k is the rate at which the inactivation rate rises after the energy density reaches that critical level (Peleg & Normand, 2004). $n(\Delta E)$ is a power parameter that indicates a deviation of the linearity of the corresponding survival curve.

The *ode45* function, which implements the Runge-Kutta method with a variable step, was used along a nonlinear least-squares solver, *lsqcurvefit*, in the MATLAB® Optimization Toolbox For the optimization process, an initial set of values for the parameters k, ΔE_c, and n was provided and at each iteration, the *ode45* solver solved the differential equation Eq. (3). The *lsqcurvefit* function was used to minimize the sum of the squared residuals (SSR) defined as the difference between the experimental data and the solution of the differential equation. The iteration was repeated until a minimum of SSR was achieved. As a result, the survival parameters k, ΔE_c, and n corresponding to the PEF process were obtained. From the critical energy density ΔE_c value a critical electric field E_c was calculated. Mean square error (MSE) (Eq. 5), and the adjusted coefficient of determination (R^2-adj), were used to indicate the adequacy of the model to describe the experimental data. R^2-adj values close to one, and a small MSE value close to zero, are indicative of good agreement between model and experimental data.

$$MSE = \frac{\sum(y_{pred} - y_{obs})^2}{n_{obs} - p} \quad (5)$$

where n_{obs} is the number of observations, p is the number of model coefficients, y_{pred} is the predicted model coefficient value, and y_{obs} is the experimental data.

5.3.9 Model validation

The modeling approach developed by this study was validated in a commercial apple cider provided by a company partner. The above procedure was tested to inactivate *E. coli* O157:H7 at conditions within the original experimental range tested and following treatment combinations that resulted in the 5-log reduction standard required to the juice industry by FDA regulations. All tests were run in triplicate and the process was replicated twice. The developed modeling approach was also validated with data published the literature for orange and strawberry juices treated in a PEF system that is similar to the one used in this project but subjected to other electric field intensities, repetition rates, and residence times. In addition, the applicability of the modeling approach developed in this research was further challenged to predict microbial inactivation of juices having different conductivities, treated at other pulse widths, pulse frequencies, residence times, and in a PEF system having a different number of treatment chambers. The modeling approach prediction's capability was evaluated by calculating the absolute error between experimental and model predictions.

5.4 Results and discussion

5.4.1 Inactivation of pathogens in apple cider by PEF

Figs. 13 and 14 illustrate survival curves for *E. coli* O157:H7 and *S.* Typhimurium inoculated in apple cider after PEF processing at different treatment combinations of electric field strength and repetition rates. Statistical analyses demonstrated that the randomized block factorial design employed was significant at the 95% confidence level. When the microbial population were considered as blocks, effects of the repetition rate, the electric field strength, and the interaction between repetition rate and electric field, on the inactivation were significantly different. When the repetition rate and the electric field were fixed, the effects on inactivation of the different microorganisms were significantly different at 1000pps and 30kV/cm. By looking at each microorganism by separate at 1000pps, the results indicated that all the electric fields were significantly different for *E. coli* O157:H7. However, at the same conditions, the results suggested that for *S.* Typhimurium electric fields of 25 and 30kV/cm were not significantly different. This difference in PEF lethality possibly suggests that blocking the microorganisms in separate treatments instead of using a mixed culture cocktail was helpful in improving the precision in comparing treatment means. More discussion of the randomized block factorial design used in this work can be found in Montgomery (2013). The 5-log pathogen reduction standard was accomplished in apple cider for *E. coli* O157:H7 and *S.* Typhimurium at repetition rates of 1000 pps at 35kV/cm, 1250pps at 30kV/cm, and 1500 at 30kV/cm were applied. Thus, these selected PEF processing parameters were able to meet the 5 $\log_{10}$ reduction criteria requested by the U.S. FDA guidelines to the juice industry (USFDA, 2001).

5.4.2 Energy density as a control PEF parameter

Table 11 summarizes some of the results for the inactivation of *E. coli* O157:H7 and *Salmonella* Typhimurium achieved in the present research. The complete inactivation data are illustrated in Figs. 13 and 14. The increase of microbial reduction was associated with the increase in the pulse repetition rate and the electric field strength employed. In other words, the higher the energy density applied, the higher is the microbial inactivation, as the energy density is positively related to the pulse repetition rate and the electric field strength. Huang, Yu, Wang, Gai, and Wang (2014) also reported that PEF microbial inactivation of *Staphylococcus aureus*, *Escherichia coli* DH5α, and *Saccharomyces cerevisiae* in grape juice, increased with the energy density and with

the electric field strength for the three microorganisms. Álvarez, Pagan, Condón, and Raso (2003a) studied the influence of electric field strength, treatment time and energy density on the inactivation of *Listeria monocytogenes* in different treatment mediums. The authors reported an increase in microbial inactivation with the increase of these parameters but concluded that both energy density and electric field strength must be used to control PEF processes. The importance of the electric field strength on microbial inactivation also agrees with the results of this study. For instance, treatments at 20kV/cm resulted in similar microbial inactivation of *E. coli* and *S.* Typhimurium, but the inactivation increased at higher electric field strengths such as at 30kV/cm, which is accordance with the levels of energy density used. Similarly, Álvarez, Raso, Sala, and Condón (2003b) reported an increased inactivation of *Yersinia enterocolitica* with electric field intensity, treatment time and energy density. By investigating the influence of the energy density on the inactivation of *Y. enterocolitica* by PEF, a pathogen isolated from dairy products, the authors reported maximum electric field strength thresholds that depended on the applied energy density. In their study, the same lethality was observed beyond those thresholds. This phenomenon was also observed in the present study when 1000pps at 35kV/cm (370 J/mL), and 1500pps at 30kV/cm (408 J/mL), i.e. similar energy density levels were applied and resulted in 6.27 and 6.32 log reductions of *E. coli* and *S.* Typhimurium, and 6.38 - 6.34 log reductions of *E. coli* and *S.* Typhimurium, respectively. Possibly confirming the existence of a maximum field strength under a certain energy density level necessary to accomplish microbial reductions greater than 6-log, and also confirming that microbial inactivation is largely influenced by the energy density applied.

In the present investigation, energy densities of 60 to 120J/ml were necessary to reduce 1-log reduction of the target pathogens, which agrees with the typical range of 1 to 100J/ml discussed by Schoenbach et al. (2000). The energy densities necessary for microbial inactivation greater than 5-log are approximately in the range 340 J/ml to 408 J/ml, i.e. within the range of 100 to 400J/ml discussed by Schoenbach et al. (2000). From a processing standpoint, the energy density would extend out as an integral parameter that accounts for the electric field intensity, the treatment time, and the conductivity of the medium. Thus, the energy density seems to be a suitable processing parameter to use as a treatment intensity parameter in a continuous flow PEF process (Toepfl et al., 2014).

Table 11. The impact of energy density in the log reduction of pathogens exposed to PEF:

f (pps)	E (kV/cm)	ΔE (J/mL)	Log reduction *E. coli* O157:H7	Log reduction *S.* Typhimurium
500	20	60.4	$1.24^{a,A}$	$1.19^{a,A}$
	30	135.9	$2.66^{b,B}$	$2.21^{a,b,B}$
1000	20	120.8	$1.59^{a,A}$	$1.14^{a,c,A}$
	30	271.7	$4.19^{c,C}$	$3.25^{d,D}$
1500	20	181.1	$2.06^{a,b,A}$	$1.41^{a,c,A}$
	30	407.6	$6.38^{d,E}$	$6.34^{e,E}$

[a]Different lowercase letters within a column are significantly different ($p<0.05$)
[A]Same uppercase letters within a row are not significantly different ($p>0.05$)

5.4.3 Modeling inactivation curves

In the present study, survival curves are plotted as the $\log_{10}$ of the survival fraction against the energy density, which accounts for both the number of pulses and the electric field strengths. As shown in Figs. 13 and 14, *E. coli* O157:H7 and *S.* Typhimurium survival curves did not follow log-linear inactivation curves in any of the applied treatments. An upward concave shape was observed for 500 and 750 pps repetition rates suggesting that the less resistant microbial population was inactivated sooner at the lower energy densities, and that it became more difficult to inactivate more resistant survivors when these repetition rates were applied. The downward concave shape detected in the range of 1000 1500 pps repetition rates suggested that regardless the populations are not able to resist higher pulse repetitions at the different electrical strength used. For instance, by increasing the energy density at those repetition rates though increases in the electrical field strength, accumulated damage decreased the microorganisms' resistance to the treatment due to the additional specific energy applied, and, therefore, the rate of the bacterial inactivation progressively increased. Thus, first-order kinetics was not suitable and therefore, not used in the work. The model chosen to account for these features was the Peleg non-linear model (Eq. 3). Literature has shown the suitability of this model to capture the inactivation data curvature in a variety of thermal (Campanella & Peleg, 2001; Chen, Campanella, & Corvalan, 2007; Peleg, Normand, & Corradini, 2005; Valero, Cejudo, & García-Gimeno, 2014) and non-thermal (Chen,

Campanella, & Barbosa-Cánovas, 2012; Mendes-Oliveira, Jensen, Keener, & Campanella, 2019) treatments.

As illustrated in Figs. 13 and 14 the model chosen to describe the inactivation curves was able to accurately describe the distinct experimental survival shapes obtained from treatments using combined processing conditions.

The MATLAB® functions (*ode45*) and *lsqcurvefit* were respectively used to solve the differential equation Eq. (3), and to find the parameters k, ΔE_c, and n that best describe the experimental data. The parameters obtained for both pathogenic microorganisms are illustrated in Table 12, which includes MSE and R^2-adj values. The goal was to demonstrate a modeling approach that would consider the energy density parameter and used it to describe the inactivation kinetics of PEF treatments. The value of the critical energy density necessary to enhance inactivation can be used to calculate the critical electric field that would promote bacterial inactivation from Eq. (1).

The uniqueness of the modeling approach developed in this study is its ability to group the effect of the repetition rate f, and the electric field strength E, into the inactivation rate. This would simplify the analysis since that both PEF process variables (E and f) may provide inactivation of the microbial cells. Thus, the critical energy density parameter obtained by the optimization procedure enabled the calculation of the critical or threshold electric field strength beyond which microbial inactivation is enhanced (Table 12). When applying lower repetition rates, greater critical electric field strength needs to be applied to inactivate targeted pathogens. Conversely, when applying greater repetition rates, a lower critical electric field strength can start the inactivation process. As shown on Table 12, the critical electric field for *E. coli* O157:H7 and *S.* Typhimurium were in range of 24-25kV/cm for the 500pps, 21-22kV/cm for the 750pps, 17-21 kV/cm for the 1000pps, 17-18kV/cm for the 1250pps and 16-17kV/cm for the 1500pps. Like results obtained in this study, García et al. (2009) also observed that the number of pulses to inactivate 1 $\log_{10}$ cycle of *E. coli* O157:H7 in apple juice decreased with the increase of the electric field intensity. This observation can be translated to the critical electric field concept, where is shown that by decreasing the number of applied pulses requires a higher critical electric field intensity for effective microbial inactivation.

As discussed above, Eq. (4) assumes that the inactivation rate is approximately zero when the applied energy density is less than a critical value, and beyond that, the inactivation rate would

follow a linear increase relationship. This assumption is consistent with results from Castro, Barbosa-Cánovas and Swanson (1993) who reported that above a critical electric field strength the microbial inactivation rate increases linearly with the applied electric field. The use of the inactivation rate as a function of the energy density described by the log-logistic relationship (Eq. 4) is consistent with the electromechanical theory. The electromechanical theory states that when the external electric field is increased, the transmembrane potential also increases because free charges of opposite polarity accumulates at the two membrane side surfaces (Pagán & Mañas, 2006). The attraction between these charges gives rise to compression which decreases the membrane thickness leading ultimately to pore formation (Castro et al., 1993). Jayaram, Castle, and Margaritis (1992) explains that if the applied electric field is present for longer time or exceeds the critical field intensity ($E > E_c$), the number of pores becomes larger and also pores with larger size are formed. Beyond that critical stage, membrane breakdown occurs and is associated with the mechanical destruction of the cell (Castro et al., 1993; Jayaram et al., 1992).

Table 12. Survival parameters to describe inactivation data for *E.coli* O157:H7 and *S.* Typhimurium inoculated in apple cider by PEF:

Pulse frequency	Peleg−log-logistic approach (Eq.3 and Eq.4)											
	Survival parameters *E.coli* O157:H7				Statistical performance		Survival parameters *S.* Typhimurium				Statistical performance	
(pps)	k(J/mL)$^{-1}$	ΔE_c (J/mL)	E_c (kV/cm)	n	R^2-adj	MSE	k(J/mL)$^{-1}$	ΔE_c (J/mL)	E_c (kV/cm)	n	R^2-adj	MSE
500	0.01	90.53	24.49	0.44	0.82	0.0324	0.01	92.40	24.74	0.42	0.99	0.0010
750	0.01	106.43	21.68	0.43	0.93	0.0204	0.01	100.54	21.07	0.39	0.99	0.0003
1000	0.01	89.75	17.24	0.41	0.99	0.0037	0.01	130.41	20.78	0.43	0.98	0.0276
1250	0.01	107.91	16.91	0.44	0.99	0.0064	0.01	151.25	20.02	0.44	0.97	0.0361
1500	0.01	126.45	16.71	0.46	0.99	0.0033	0.02	124.35	16.57	0.08	0.99	0.0004

5.4.4 Model validation

To test the prediction capability of the developed model, same pathogen and same PEF system but different PEF process conditions and different juice were used. Commercial apple cider inoculated with *E. coli* O157:H7 was tested at conditions of 1000pps at 35kV/cm and 1500pps at 30kV/cm. The model parameters reported in Table 12 were used in Eq. (3) to calculate the inactivation rate. The electrical conductivity of the commercial apple cider used was of 1558 μS/cm, pH 3.83, and solid content 12.0°Brix. The error between the experimental and predicted values is reported in Table 13.

Table 13. Validation results of *E. coli* O157:H7 inoculated in a commercial apple cider with electrical conductivity of 1558.4 μS/cm

PEF treatment conditions	Experimental values	Modeling approach inactivation prediction	|Error|
1000pps / 35kV/cm	4.03	4.48	0.45
1500pps / 30kV/cm	4.84	4.29	0.55

Validation results are shown in Table 13. The error between the experimental and predicted values suggest that the developed model provides reasonable predictions of the inactivation of *E.coli* O157:H7 in the commercial apple cider having a different conductivity than the one used to build the model. The error between experimental values and model predicted values was 0.45 when 1000pps at 35kV/cm was employed, and 0.55 when the 1500pps at 30kV/cm condition was tested. The low errors indicate that the model results in reasonable predictions.

The developed model was further validated using the data published in the literature (Gurtler et al., 2010, 2011). Those PEF experiments were conducted in a different laboratory with the same laboratory-scale pulsed electric system (OSU-4H). As shown in the literature, the electric field intensities were 11.75, 19.31, 23.86, 28, and 31.72 kV/cm for orange juice, and 18.6 kV/cm for strawberry juice; pulse repetition rates for both juices were 800 pps. Therefore, the parameters obtained from the 750pps and 1000pps (Table 14), which is close to 800 pps, were used to predict the inactivation of *E.coli* O157:H7 in orange and strawberry juices. The comparison of predictive values and published data from Gurtler et al. (2010, 2011) are listed in Table 14. The low values of the error confirm the suitability of the model to predict microbial reduction in juices subjected

to PEF. The model was able to account for the different processing parameters applied such as electric field strength, repetition rate, pulse width of 2.6μs, juices' conductivity, as well as the residence times explored by Gurtler et al. (2010, 2011).

It is important to point out that conductivity is a factor that plays a significant role in the PEF treatment effectiveness. Generally, PEF treatments are more effective in foods with low electrical conductivity (Jin et al., 2015). The difference between the electrical conductivity of the medium or juice, and that of the microbial cytoplasm increases the flow of ionic substances across the membrane, which as a result is weakened, thus becoming more susceptible to PEF treatments (Barbosa-Cánovas et al., 1999). Other juices with different conductivities such as apple, watermelon, and melon were also explored to validate the modeling approach developed in the present study. These juices were processed also with the OSU-4H system but composed of 8 treatment chambers, different flow rates, pulse frequency and pulse width, than those used in this study. The optimized survival parameters, juices conductivities, along with the PEF treatment conditions from the literature (Mosqueda-Melgar et al., 2008a, 2008c) to achieve a target inactivation of *E. coli* O157:H7 are reported in Table 15. The small error between experimental and predicted log inactivation confirms the good prediction capability of the model developed in this study. It also confirms that is necessary a critical energy density to enhance microbial inactivation. From that, a critical electric field can be determined from the model. The critical E_c for melon was higher than that of apple and watermelon juices. This is explained because of the high conductivity of melon juice, which would decrease the PEF efficacy to inactivate *E. coli* O157:H7 in melon juice. As explained previously, juices with greater electrical conductivity tend to impact negatively the inactivation of microorganisms (Jin et al., 2015). As shown in Table 15, the n parameter was treated as a constant coefficient not necessarily a function of energy density; whereas the k rate was greater for apple juice, which is an indication of the highest inactivation achieved for that juice as compared to melon and watermelon.

The main advantage of the proposed modeling approach, which is based on the selection of the energy density parameter, is that accounts for others PEF processing parameters such as the electric field strength, the pulse repetition rate, the pulse width, the residence time as well as the samples electrical conductivities. Furthermore, a critical electric field can be calculated (from the critical energy density). Although the present model was developed and validated from data using square wave pulses and co-field continuous treatment chamber configurations, this do not

undermine the value of this study or its general application. The electric field intensity for coaxial chamber design, for instance, can be calculated if the outer and inner radii of the electrodes are known (Altunakar & Barbosa-Cánovas, 2011). If exponential decay pulses are used, the average or effective voltage (approximately 37% of peak voltage) can be used for calculating electric field intensity; and the effective pulse width (the time until the electric field decreases to 37% of peak voltage) can be used for the model. Jin, 2017; Toepfl, et al., 2014). Thus, the energy density can be calculated for other situations as well (Altunakar & Barbosa-Cánovas, 2011; Jin, 2017; Martín-Belloso & Soliva-Fortuny, 2011; Zhang et al., 1995) and used as a control PEF parameter to study PEF processes.

A 5-log inactivation of target microorganisms is a fundamental requirement for FDA approval. The experimental results and the model developed here was able to accurately estimate the conditions that meet this requirement. The approach is suitable for industrial applications because incorporates juice properties, the required energy demand, the ideal pulse repetition rate, and the field strength in the design of the PEF process. Furthermore, the approach should assist the food industry in determining appropriate PEF conditions for the inactivation of pathogenic cells in order to prevent the incidence of costly food-associated outbreaks.

As discussed in the previous studies (Jin, 2016; Jin et al., 2015), PEF processing technology is different from other non-thermal processing, as so many parameters are involved in a PEF system design and operation. The complexities of PEF treatment are a challenge for potential users of PEF pasteurization, especially for those in industry, who have less research experience with this technology, which is one of the obstacles for the commercialization of this technology in food pasteurization. By grouping the parameters, the mathematical simulation or modeling provides the base for the development of user-friendly software applications or interfaces, with which it could achieve “one button” operation for industry operators wherever PEF processing is needed.

Table 14. Validation results reported in the literature of *E. coli* O157:H7 inoculated in other juices treated with the OSU-4H PEF system at other pulse repetition rate, pulse width, residence time and electric field strengths:

Juice		PEF treatment conditions						Data	Modeling approach log reduction		|Error|		Data source
	Conductivity (μS/cm)	Pulse frequency (pps)	Pulse width (μs)	Number of chambers	Residence time (μs)	Electric Field (kV/cm)	Energy density (J/mL)	log reduction	Parameters from the 750 pps	Parameters from the 1000 pps	Parameters from the 750 pps	Parameters from the 1000 pps	
Orange	4290	800	2.6	6	6024.397	11.72	44.30	0.49	0	0	0.49	0.49	Gurtler et al., 2010
						19.31	120.27	1.90	1.42	1.51	0.48	0.393	
						23.86	183.62	2.97	2.18	2.43	0.789	0.514	
						28	252.87	3.76	3.14	3.65	0.617	0.106	
						31.72	324.53	4.10	4.31	5.16	0.214	1.057	
Strawberry	4200	800	2.6	6	12048.79	18.6	218.49	3.09	2.81	3.23	0.282	0.142	Gurtler et al., 2011

Table 15. Model parameters from published data in the literature and the validation results of *E. coli* O157:H7 inoculated in other juices treated with the OSU-4H PEF system having 8 chambers, system set at other pulse repetition rates, pulse width of 4 μs, residence times, and electric field strength of 35kV/cm:

Juice		PEF treatment conditions						Optimized model parameters				Data	Modeling approach	|Error|	Data source
	Conductivity (μS/cm)	Pulse frequency (pps)	Pulse width (μs)	Number of chambers	Residence time (μs)	Electric Field (kV/cm)	Energy density (J/mL)	ΔE_c (J/mL)	k (J/mL)$^{-1}$	n	E_c (kV/cm)	log reduction	log reduction		
Apple	2180	180	4	8	8087.926	35	4205.028*	1980	0.0012	0.36	24.017	4.17	4.174	0.0043	Mosqueda-Melgar et al., 2008c
Melon	6020	193	4	8	276716.056	35	12603.008	8000	0.0008	0.20	27.885	~3.8	3.820	0.0199	Mosqueda-Melgar et al., 2008a
Water melon	3540	200	4	8	262812.5	35	7293.993	3800	0.0008	0.28	25.263	~3.5	3.577	0.0771	Mosqueda-Melgar et al., 2008a

*this value was obtained after multiplying by 33.8 which was retrieved from Mosqueda-Melgar et al. (2008c) in which the authors reported the treatment time multiplied by this value.

5.5 Conclusions

Non-thermal technologies are of growing interests in food engineering. The primary intent of this study was to develop a modeling approach to describe PEF treatment by establishing a correlation between the PEF processing parameters and the inactivation characteristics of pathogens that have been associated with juice-related outbreaks. The developed model enables the calculation of microbial inactivation rate as a function of the energy density that groups key PEF processing parameters, such as electric field strength, treatment time, and sample's conductivity.

The model was constructed with the assumption that the death rate is not constant and depends on the instantaneous PEF processing conditions. Thus, the approach estimates inactivation parameters under more realistic processing conditions. Among the survival parameters obtained with the model, a critical energy density can be calculated, and the PEF processing conditions to meet the 5 $\log_{10}$ reduction criteria can be obtained.

The model was validated by predicting inactivation rate on other apple cider samples having different conductivities but treated under conditions in the same range than those tested in the present study. Furthermore, the approach was validated with data from the literature of juice samples treated with other PEF systems, having different number of treatment chambers and set at other electric field intensities, pulse repletion rates, pulse width, and residence times. Both validations demonstrate that the developed model well predicts the data from different sources.

The modeling approach represents a major improvement in non-thermal processing calculations not only because replaces the widely used first-order kinetic model for applications where more realistic non-lineal experimental survival curves are obtained, making markedly contributions to the current knowledge of predictive models for survival curves of microorganisms exposed to PEF, but also groups the PEF processing parameters in an integral energy density parameter, which significantly simplify the PEF processing parameters and treatment conditions, hence, promote the commercial application of the technology.

Author's contribution

Gabriella Mendes C. de Oliveira conceived the idea of this study, the experimental design, conducted the experiments, interpreted the data, wrote the code, ran the model, analyzed the output data and wrote the manuscript. Osvaldo H. Campanella supervised the modeling analysis, Tony Z.

Jin supervised the PEF pasteurization tests and the microbiological experiments. The authors declare that there is no conflict of interest.

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5.6 Figures

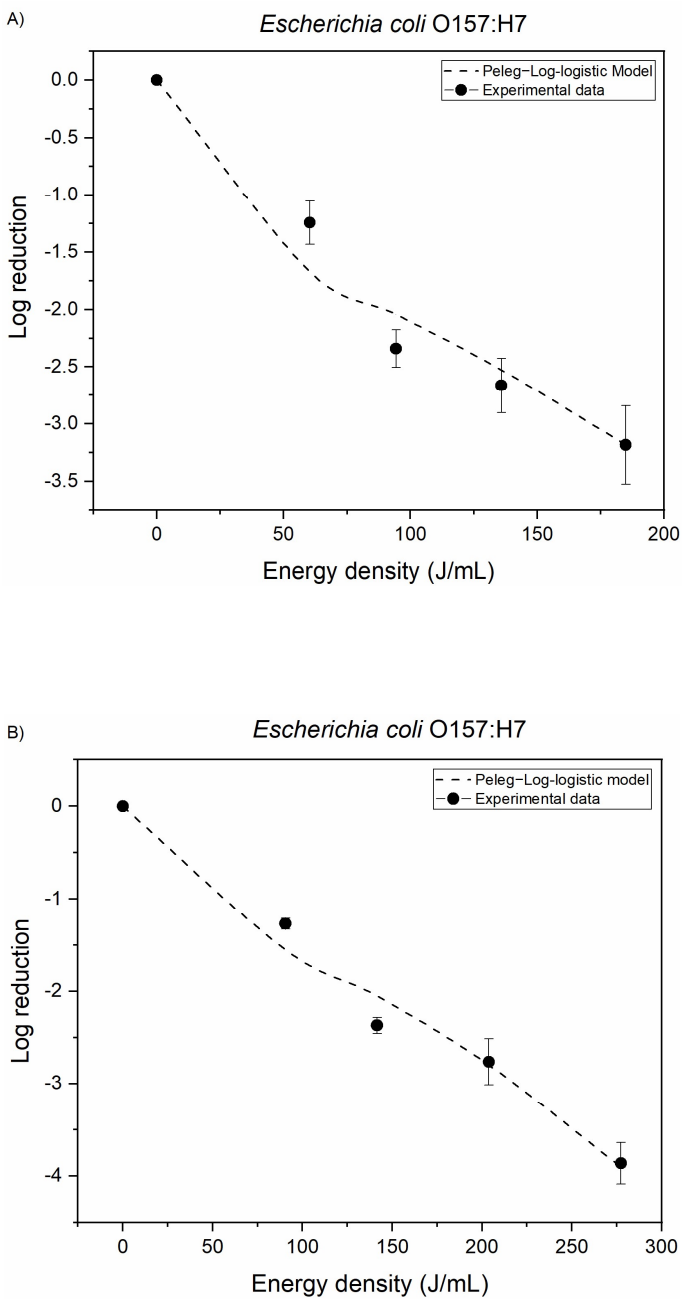
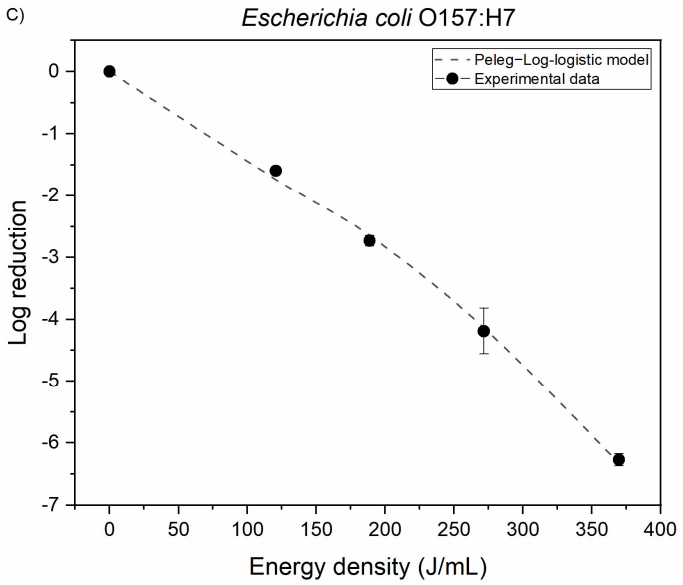


Fig. 13. Survival curves of *E. coli* O157:H7 in apple cider during PEF processing at: A) 500pps *x* 20, 25, 30, 35kV/cm; B) 750pps *x* 20, 25, 30, 35kV/cm; C) 1000pps *x* 20, 25, 30, 35kV/cm; D) 1250pps *x* 20, 25, 30kV/cm; E) 1500pps *x* 20, 25, 30kV/cm. Dashed line represent the Peleg−log-logistic approach and dots represent the data.

Fig. 143. Continued



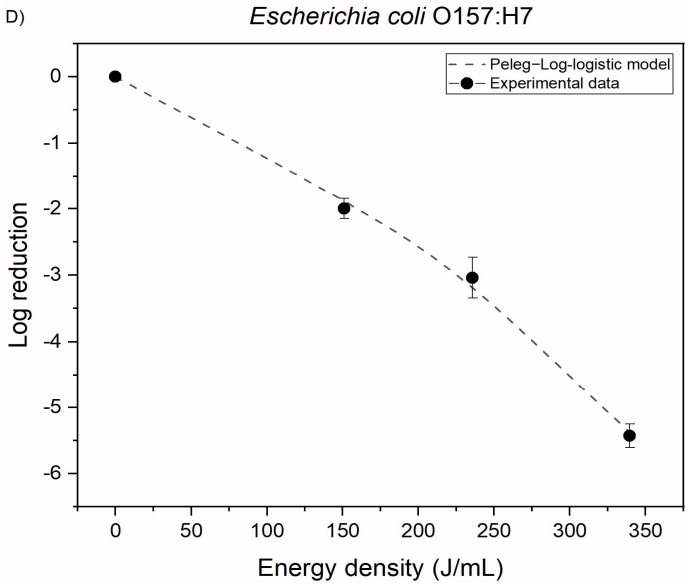
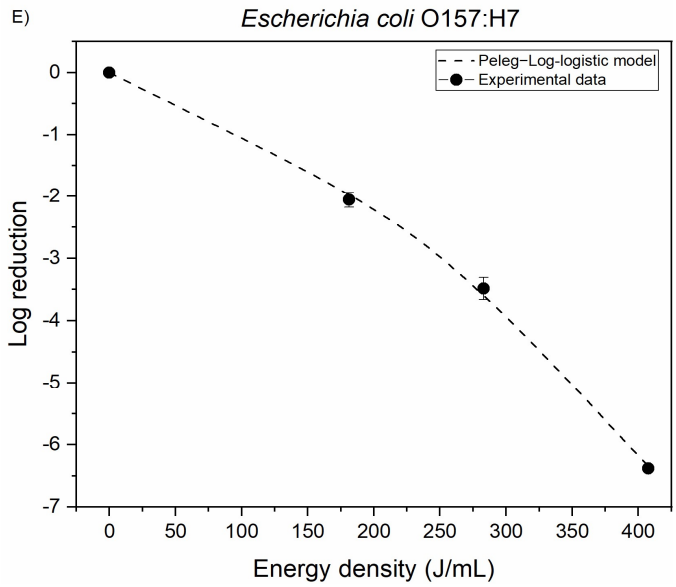


Fig. 13. Continued



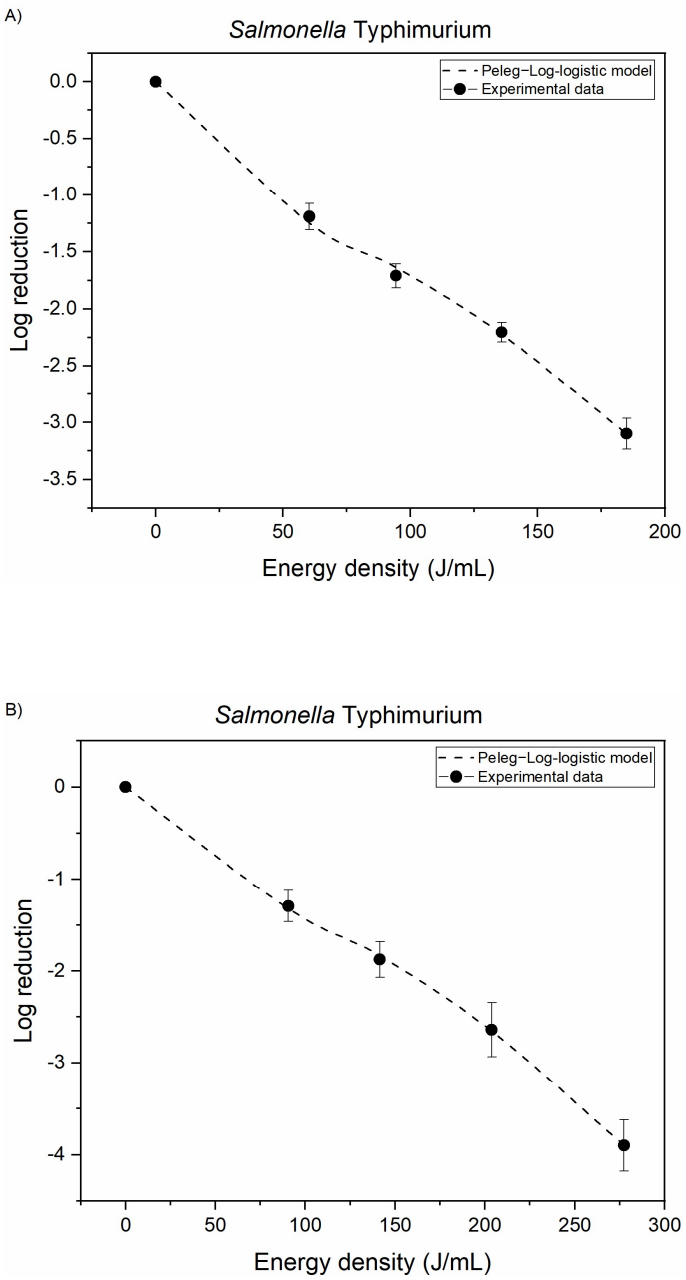
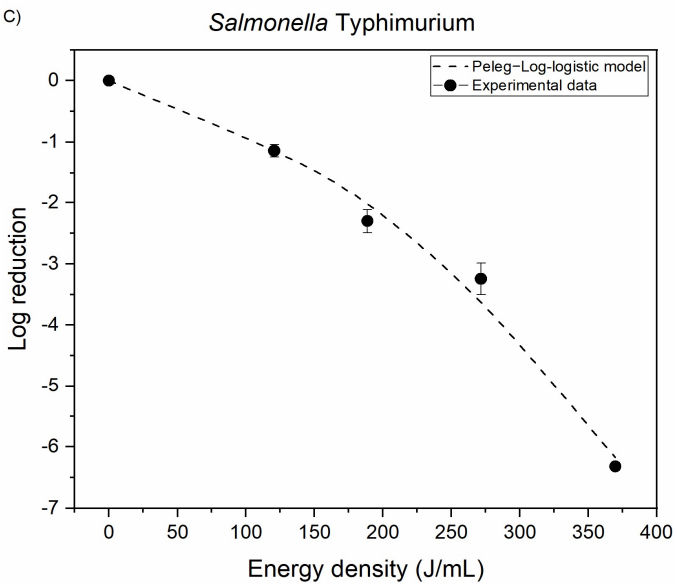


Fig. 14. Survival curves of *S.* Typhimurium in apple cider during PEF processing at: A) 500pps *x* 20, 25, 30, 35kV/cm; B) 750pps *x* 20, 25, 30, 35kV/cm; C) 1000pps *x* 20, 25, 30, 35kV/cm; D) 1250pps *x* 20, 25, 30kV/cm; E) 1500pps *x* 20, 25, 30kV/cm. Dashed line represent the Peleg−log-logistic approach and dots represent the data.

Fig. 14. Continued



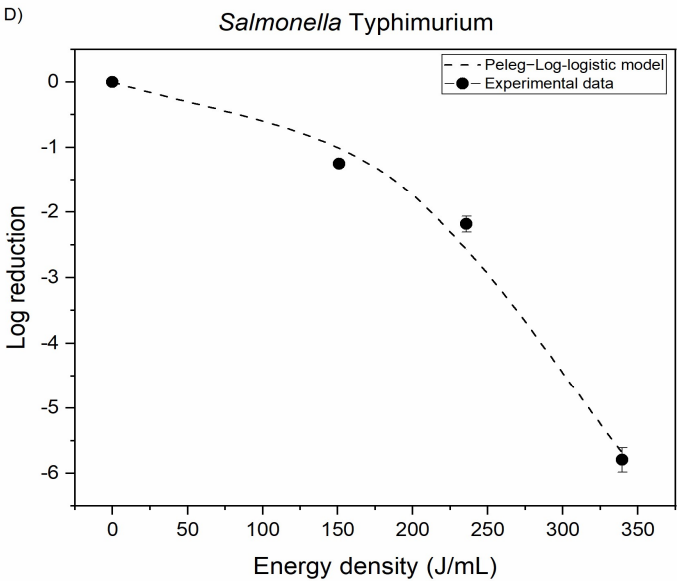
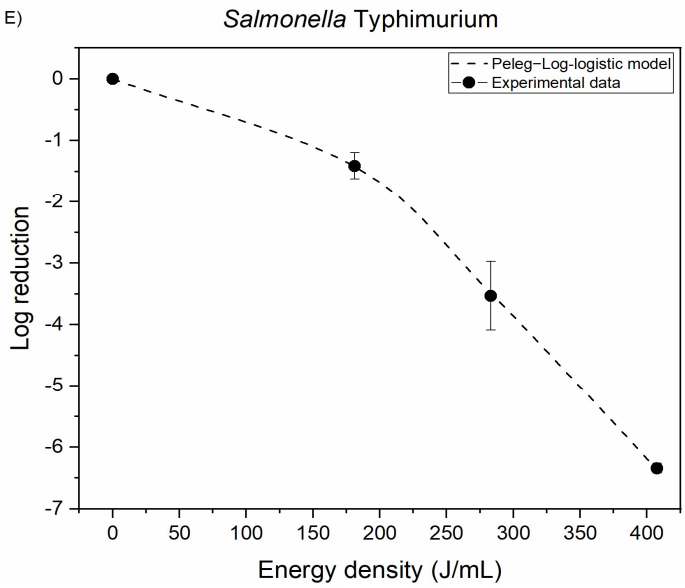


Fig. 14. Continued



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CHAPTER 6. SUMMARY AND RECOMMENDATIONS

6.1 Summary of the Dissertation

Mathematical models compromise and are associated to a wide range of applications that can assist the food industry to predict, control and improve system performance, which will ultimately aid in the management of food safety programs. The literature review chapter highlighted current models employed to describe the inactivation of microorganisms under thermal and non-thermal food processes investigated in this dissertation. The chapter also discusses in detail gaps and opportunities that are lacking in this area of research as reported in the literature. The main conclusion that can be extracted from the review is that mathematical models describing the inactivation of microorganisms found in the literature are far to be complete.

The food industry is constantly trying to adapt to consumer demands that come with the inherent expectation that foods are free from potentially harmful microbes, but that are considered fresher and more natural. Thus, growing interests in alternative methods for food processing opens up the opportunity to explore technologies such as microwave heating, pulsed electric fields, and cold plasma, to meet these tight demands. In regards to food safety, mathematical modeling is a valuable tool for quantitative characterization of survival curves and for supporting food safety decisions considering these novel technologies.

Non-thermal plasma or cold plasma has emerged as an innovative technology for microbial inactivation in the last two decades. The abundant reactive species generated during cold plasma treatments have been considered the main process parameter affecting the inactivation of vegetative and spore-forming organisms. The extreme resistance of spore cells often requires harsh (e.g. heat) treatments which in turn are likely to cause adverse effects on the freshness, nutrition and quality of foods. Because of the relatively mild conditions of cold plasma processes and the generation of highly oxidative gas species, the technology offers the opportunity to inactivate spore-forming organisms that present risks in foodborne diseases or food spoilage. Quantitative approaches are essential elements of microbial food safety and are required for regulatory approval. Concerning quantitative approaches used in the modeling of cold plasma treatments, one of the major gap is the assumption that the concentration of

reactive species is constant, which is far from real conditions occurring during plasma processing. The other aspect that is not taken into account is the fact that, despite survival curves are visibly non-linear, first-order kinetics is used and, unfortunately, this shortcoming does not aid in the design of plasma processes in practice. Chapter 3 of this dissertation proposes a modeling approach that jointly modeled the time-varying concentration of a major reactive specie and the kinetics of microbial inactivation. The approach enabled the determination of a critical gas concentration of that specie, which would provide a threshold value to be employed in order to achieve significant microbial inactivation. Thus, from a practical standpoint, the modeling approach provides a useful and practical relationship between of the inactivation characteristic of resistant spores with realistic plasma processing factors. Although the cold plasma technology has not gained status for its use in the food industry, the modeling approach developed in this dissertation should empower the HACCP system used with this technology to determine the critical processing variables that play a key role in plasma processing.

Recent advances in the use of emerging technologies for the inactivation of foodborne pathogens with minimal changes in nutritional attributes paved the way for the exploration of microwave heating for food pasteurization, as opposed to the exclusive use of convective heating. Microwave pasteurization may mimic similar processing variables as those used in traditional pasteurization protocols, but heating times are shorter and heat penetration is significantly higher. Furthermore, temperature profile in microwave processing is far from constant. Therefore, typical nonisothermal conditions of microwave heating should be included in the modeling analysis. The major gap in the literature, in terms of microbial inactivation modeling under microwave heating, was to assume first-order models to describe inactivation curves, which are far from linearity. More importantly, a common flaw reported in the literature was to ignore the temperature history of microwave processes and assume some arbitrary reference temperature to calculate D, z and F values so that the microwave process could be related to isothermal conditions. Chapter 4 of this dissertation addresses this flaw by proposing a modeling approach that is able to relate the more realistic time varying temperature profile to the microbial inactivation kinetic by using a nonisothermal rate model. Two models, the Arrhenius and the logistic models, were used to describe the rate of inactivation and the dependence of the model parameters with the temperature and their

prediction capacity was compared and validated for other treatment conditions and samples. It was clear that the Arrhenius model failed to predict inactivation at different conditions. Other major contribution of the modeling approach used is the finding of a critical temperature above which significant microbial effects are significant. Once the food industry tests this approach in its liquid products, a target temperature can be determined. This temperature should assist the food industry in the design of microwave process as well as in the decision-making process to define which variables should be evaluated to ensure that pasteurization is effective. Finally, the modeling approach will further assist in process validation to ensure that the 5-log reduction criteria requested by the FDA HACCP guidelines are met.

The growing interests of Pulsed Electric Fields (PEF) in food engineering is due to the large body of evidence that PEF is able to inactivate microorganisms. While mathematical models have been explored to describe the inactivation curves under PEF, none of the studies discussed in the literature review chapter were able to incorporate all the PEF variables into the modeling analysis. In some cases, the log reduction was plotted either against treatment time, or electric field strength, or specific energy, and the Weibull model was used to fit the experimental data. A good fit of the inactivation curve is important to estimate model parameters, and the shape of the curves were well described by the Weibullian shape parameter. In some cases, polynomial equations were also proposed to infer the contribution of variables such as E^2 and Et^2 in the modeling analysis, but their practical significance in the design of PEF was not discussed. The deficiency of these currently methods opens up the floor for development of more realistic modeling approaches that should better assist the design of PEF pasteurization protocols. Chapter 5 of this dissertation proposed a modeling approach based on the energy density parameter to describe the PEF pasteurization of fruit juices. It was hypothesized that microbial death is non-constant and depends on the energy density applied. From a calculated critical energy density, a critical electric field was calculated for processing conditions such as pulse repetition rates, which range from 500 until up to 1500pps. This hypothesis agrees with a theoretical critical electric field parameter, whose existence has been discussed for many years in the PEF literature. Working with the energy density applied, the modeling approach proposed in this dissertation incorporated all the PEF process variables into the description of the inactivation curves at the same time. In other words, the electric field strength, the pulse repetition rate, the pulse width, the residence time, and the sample's conductivity were jointly incorporated in the microbial inactivation kinetics for accurate

predictions. The proposed approach was validated in other samples and also in data published in the literature for other juices. For the first time, such a model has been developed and validated, which proves its usefulness and its rigorous framework for the design of PEF processes. Finally, in addition to its striking contribution to predictive models for PEF, the proposed approach will also assist the food industry by using the approach to support food safety decisions. For instance, in determining which parameters must be considered to ensure the safe production of foods, or in determining which factors need to be considered in the control of PEF pasteurization processes.

The use of mathematical models to describe inactivation curves becomes powerful when process variables are linked to microbial kinetics. Although the assignment is not simple, when microbial kinetic parameters are estimated using realistic process factors that actually correlate with the behavior of microorganisms in food, the result is a safe inference for process design. This dissertation successfully completed this assignment and contributed significantly to the current knowledge of predictive models for microbial inactivation. The examples explored in this dissertation that correlated important process parameters with microbial inactivation – e.g. the concentration reactive species of cold plasma, temperature history of microwave heating, and the energy density of PEF treatments – showed that mathematical modeling will realistically assist the food industry in the design, control and optimization of nonisothermal and non-thermal food processes.

6.2 Recommendations for future work

The dissertation was able to fulfill its objective of developing a flexible modeling approach capable of incorporating the process variables in the description of microbial inactivation, whether that is subjected to nonisothermal or non-thermal food processes. All of the phases of the modeling cycle were satisfied, from the planning of the experimental design, the collection of the experimental data, the description of model incorporating relevant factors and its prediction capacity confirmed in untested combination of factors, even its complete validation in more complex systems. But still, some questions can be further investigated in future research.

For cold plasma, the empirical modeling approach developed to describe the generation of ozone is not yet related to plasma physics. Once the random production of reactive species is modeled, the equation, or its algorithm, can be linked to the approach proposed by this dissertation to build a more mechanistic method.

For microwave heating, the modeling approach was built on juice samples since microwave heating is already being used successfully for fluid products in continuous systems. For instance, in recent continuous systems that focus the microwave energy inside cylinder tubes which minimizes any non-uniformity in the heating of fluid products. (This is an interesting advantage for quality attributes, see for example the company SinnovaTek which sells microwave systems for continuous fluid with flows ranging from 2-100 gallons per minute. In one of its open access report, the company also shows the retention of color, nutrient and aroma in a variety of smooths and purees treated by continuous flow microwave processing). But even with the method of modeling developed in this dissertation, the door for validation of the proposed model for solid foods or multi-food components, that may include particulates, solid, or viscous materials, is still open. For such complex systems, uneven temperature distribution, resulting in hot or cold spots in microwave-heated products may compromise the efficacy of pasteurization or sterilization protocols. Given the wide range of food products along with their associated chemical/physical singularity, or their associated pathogens of concern, no single universal model will likely meet all the requirements. Therefore, future research should tackle each case and its uniqueness separately in order to build a modeling approach that can assist decision-making for food safety management. Nevertheless, it is important to provide the industry with realistic modeling approaches that incorporate the history and evolution of the lethal variables into microbial prediction. It is believed that the modeling approach proposed by this dissertation did just that.

For PEF processes, the mild temperatures that may be associated to PEF are below those used in the traditional thermal pasteurization still make the technology of practical interest, especially with the reduction of the negative impact that high temperatures have on food quality. The modeling approach proposed in this dissertation assumed that if any thermal effects existed they were accounted for in the calculation of the energy density. Future modeling research could separate any thermal from the electric field effects, for example in terms of potential effects on the microbial membrane. Other future modeling research could relate the critical electric field with the size of the bacterial cells. Models like those would be based on theory rather than practical processes, but still they could be useful to develop.

In addition, future research may evaluate other intervention technologies and use the ideas constructed in this dissertation as a reference or as a starting point to build more realistic modeling approaches for those technologies as well.

Last, but not least, future research should assess the conditions that are able to meet the 5 log reduction criteria in terms of their impact on the quality and nutritional attributes. In other words, the optimized food safety conditions should also be assessed in a food quality perspective.

VITA

Gabriella Mendes Candido de Oliveira was born in Minas Gerais (Brazil), to parents whose farm roots reflect her passion for food and agriculture. To earn her BS degree, she was accepted to prestigious Brazilian Universities: University of São Paulo (USP), São Paulo State University (UNESP), and the Federal University of Visçosa (UFV), colleges that offer tuition-free education and are more prestigious than the private institutions. She chose USP, the leading institution in Brazil for research and teaching, to earn her bachelor's degree in Food Engineering. At USP, she cherished all the opportunities she had to learn more, working her way through college with scholarship awards to conduct research. After working as trainee in quality assurance at Anheuser-Busch InBev (AMBEV, Brazil), her passion for food safety really increased. The main project that she was enthusiastic about was revising the HACCP plan, which was triggered by a plant modification. She prepared the flow diagram by examining every step in the process and making the diagram more accurately representing the beer brewing process. Besides that, she lectured employees on GMP, sanitation, and HACCP. This experience made her more curious about biological hazards, the importance of the preventive, and process controls. This curiosity instilled her desire to pursue a Ph.D. degree in Biological and Food Process Engineering. Thus, Gabriella decided to go for graduate school. Her dream: Purdue University, the number #1 in Agricultural and Biological Engineering in the United States. After a pre-selection process by a nonprofit organization affiliated to Harvard University, Gabriella wrote and submitted a research proposal to CAPES Foundation and received a Fellowship Grant of more than $200.000. At Purdue, Gabriella is the recipient of the 2019 Outstanding Ph.D. Student Award at the ABE Department, a consequence of Gabriella's commitment to her academic performance. In addition, she was a graduate teaching assistant for the classes ABE 308 (Heat and Mass transfer) and ABE 303 (Application of Physical Chemistry to Biological Processes) where her teaching skills leading tutorial sessions were highly acknowledged by the students. Gabriella also contributed to the Department as the recruitment chair (ABE-GSA 2016/17), and by serving in other roles. Besides her collection of high marks and talent as a researcher, her career-driven personality prompted her to pursue a research collaboration with Dr. Tony Jin at the U.S. Department of Agriculture. In 2018, she worked at the USDA-ARS-ERRC as a visiting scientist in projects related to inactivation of pathogens by non-thermal food process technologies.