CHAPTER 8

Impact of irradiation on the microbial ecology of foods

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8.1 Introduction

Irradiation is a process of exposing a food item to certain types of radiation energy to induce desirable changes. Radiation is defined as energy moving through space in invisible waves. Radiant energy has different wavelengths and degrees of power (Josephson and Peterson, 2000). Figure 8.1 depicts the electromagnetic spectrum. The sepectrum has two major divisions including non-ionizing radiation and ionizing radiation.

Radiation that has energy to move atoms in a molecule around or cause them to vibrate, but not enough to remove electrons, is referred to as "non-ionizing radiation". Near ultraviolet (UV) light, visible light, infrared, microwave, and radio waves are all examples of non-ionizing radiation.

Radiation that has enough energy to remove tightly bound electrons from atoms, thus creating ions, is called "ionizing radiation". It has a higher frequency and shorter wavelength than non-ionizing radiation. Far UV light, X-rays, and gamma rays are regarded as ionizing radiation.

8.2 Ionizing radiation

The ionizing radiation of interest in food preservation is known as "irradiation". Irradiation is capable of destroying harmful bacteria, viruses, or insects that might be present in the food (Sommers and Fan, 2006). In comparison with other food processing techniques, food irradiation is relatively well studied. Although the safety and benefits of food irradiation have been thoroughly documented, the commercial application of the process has been prevented due to some misconception by the general public on its safety (ICGFI, 1999). The use of irradiation has been approved for different types of foods in over 55 countries worldwide (Farkas and Mohacsi-Farkas, 2011). The list of irradiated products is limited to spices, herbs, seasonings, some fresh or dried fruits and vegetables, seafood, meat and meat products, poultry and egg products (CFS, 2009).

The radiation dose (D) is the quantity of radiation energy absorbed by the food as it passes through the radiation field during processing. It is measured using a unit called the Gy (ICGFI, 1999). Irradiation can be used to sterilize food products at levels above 10 kGy. In the range of 1–10 kGy it can be used to pasteurize food by eliminating a significant number of microorganisms including those of publich health sigificance. In some products it can be used as an insect disinfestation treatment (<1 kGy) and a sprout inhibitor in potatoes and onions (<0.5 kGy). It can also delay ripening of certain fruits (<0.3 kGy) and eliminate *trichinosis* in pork (<1 kGy) (Keener, 1998).

Quantitative Microbiology in Food Processing: Modeling the Microbial Ecology, First Edition.
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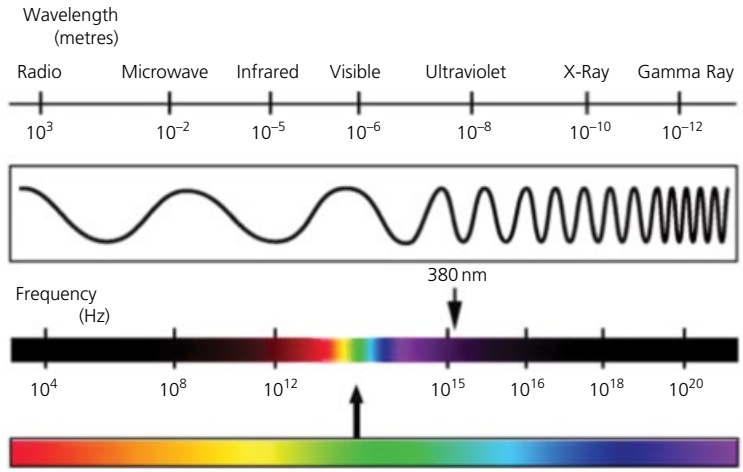

Figure 8.1 Electromagnetic spectrum (from http://www.viewzone2.com/dna.html).

The food irradiation process uses three types of ionizing radiation sources including Cobalt-60 and Cesium-137 gamma sources, electron beam generators, and X-ray accelerators. Cobalt-60 or Cesium-137 emits ionizing radiation in the form of intense gamma rays. X-ray accelerator uses an electron beam to target electrons on a metal plate. Some of the energy is absorbed and the rest is converted into X-rays. Electron beam generators produce e-beams accelerated to 99% of the speed of light and generate energies of up to 10 MeV. The electromagnetic radiations of the first two types of sources have good penetration ability, while accelerated electrons have low penetrability (Farkas and Mohacsi-Farkas, 2011).

8.2.1 Impact of ionizing radiation on food-borne microorganisms

8.2.1.1 Inactivation mechanism

Except for different penetration, the effects of electromagnetic ionizing radiations and electrons are the same in food irradiation (Farkas, 2006). The primary effect of food irradiation is produced by energetic electrons. When ionizing radiation is passing through an item, it may result in ionization (removal of an electron), dissociation (loss of a hydrogen atom) and excitation (raising the energy of a molecule to a higher energy level) types of chemical reactions in irradiated foods (CFS, 2009). The major product of an ionization reaction is reactive free radicals produced as a result of the primary effect. The secondary effects will occur when the free radicals undergo reactions such as recombination, electron capture, or dimerization (Alper, 1977). The presence of oxygen and water also have a profound effect on the radiolytic process.

The ionizing radiation generates reactive oxygen species, e.g., hydroxyl radicals and hydrogen peroxide, which result in oxidative stress in biological cells and damage cell components such as proteins, lipids, and deoxyribonucleic acid (DNA), leading to cell death (Asad *et al.*, 2004). Irradiation-induced cell death is primarily through extensive DNA damage. DNA is very sensitive to irradiation and is regarded as the main target of both ionizing and non-ionizing radiation. Irradiation of DNA causes base damage, breaking DNA strands, and cross-linking. All of these result in cell death (Alper, 1977).

The ability of microorganisms to survive upon irradiation depends on the damage produced in the cell, the number, nature, and duration of irradiation-induced chemical compounds, and

the inherent ability of cells to withstand ionizing radiation. pH, temperature, and chemical composition of the food in which microorganisms are suspended have also an important influence on the survival of microorganisms upon irradiation (Monk *et al.*, 1995).

There is an increasing public awareness of the health threat posed by food-borne pathogens. *Escherichia coli O157:H7, Salmonella, Campylobacter jejuni, Listeria monocytogenes,* and *Vibrio* are of primary concern from a public health standpoint. *Salmonella* and *C. jejuni* are usually associated with poultry. *E. coli O157:H7* has also been linked to major food-borne disease outbreaks through meat and dairy products, apple juice, water, and vegetables (Moterjami *et al.*, 2014). *Listeria monocytogenes* has been associated with dairy products, processed meats, and other foods having a relatively long shelf life under refrigeration. *Vibrio* spp. have been the causative agents in world cholera pandemics. These pathogens are sensitive to low levels of ionizing radiation (ICGFI, 1999).

8.2.1.2 Modeling inactivaton kinetics

Developement of mathematical equations describing the behavior of microorganisms under different environmental conditions (phsical, chemical, competetive) is the goal of predictive microbiology. In other words, predictive microbiology is related to the quantitative microbial ecology of foods. Mathematical models allow the description and prediction of microbial behavior under specific environmental conditions. Models can be classified as kinetic and probability models, emprical and mechanistic models, or primary and secondary models (Fakruddin, 2011). Kinetic models are considered with the rates of response (growth or dead) (e.g., log-linear, Gompertz, square root models, etc.). Probability models indicate the probability of growth. Empirical models usually take the form of first or second degree polynomials and are used in curve fitting (e.g., the quadratic response surface model of Gibson *et al.*, 1988). Mechanistic or deterministic models are developed from the theoretical bases and permit interpretation of the response in terms of known phenomena and processes (McMeekin *et al.*, 1993; Draper, 1988; Whiting and Buchanan, 1993). Primary models measure the response of the microorganism with time to a specific condition (e.g., growth, growth–decline, *D* values or thermal inactivation, inactivation–survival models). Secondary models describe how the factors of an actual growth environment affect the growth parameters (response surface, Arrhenius models, etc.) (Baranyi, 2003).

Mathematical models that allow predictions to be made of microorgamism growth or decline represent a great benefit for the food irradiation process for assesment of the safety of foods (Tomac *et al.*, 2013).

The first-order inactivation model (log-linear model) satisfactorily describes the microorganisms response to irradiation treatment and estimates the D_{10} value. The D_{10} value is the irradiation dose necessary to reduce the number of particular microorganisms by one logarithmic unit. It provides a basis for accurate estimation of inactivation doses (Adu-Gyamfi *et al.*, 2012).

The first-order inactivation model assumes that the time rate of decrease in population (inactivation rate) (dN/dt) changes linearly with respect to population, N, and the dose rate (dD/dt)(Miller, 2005), such that

$$\frac{dN}{dt} = -kN\left(\frac{dD}{dt}\right) \tag{8.1}$$

After eliminating the time variable and integrating gives

$$N = N_0 e^{-kD} = N_0 e^{-(kD/2.3)} \tag{8.2}$$

where k is the first-order inactivation constant in units of kG/y, N_0 is the initial population at the start of irradiation, and D is the accumulated dose (kGy). The D_{10} value showing the resistivity of a microorganism to irradiation can be defined as

$$D_{10} = \frac{2.3}{k} \tag{8.3}$$

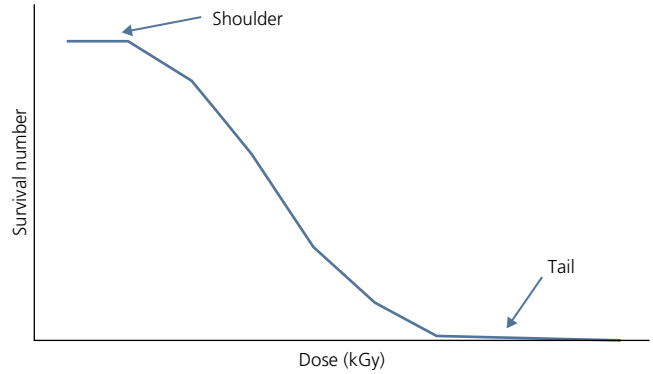

Figure 8.2 The dose inactivation curve of a typical bacterial population.

Equation (8.2) can also be expressed in logarithmic form (Tomac *et al.*, 2013), such that

$$\frac{\log N}{\log N_0} = -kD \tag{8.4}$$

In this case, the value of D_{10} is determined by calculating the reciprocal of the slope ($1/k$).

The first-order model was able to reasonably predict microbial inactivation when the irradiation dose was within certain limits. However, at low irradiation dose levels, a shoulder can be observed for some microorganisms (see Figure 8.2). The value of the first-order inactivation constant is low in the low-dose range, indicating that the organism has an effective DNA repair system. The first-order inactivation constant increased with dose and remained constant within a certain dose range. For these types of inactivation curves, two parameter models can be used to take account of the shoulder effect (Miller, 2005).

The radiation resistance (D_{10} value) of pathogenic bacteria exhibit differences in various media. It also varies between species. The order of radiation resistances of the common vegetative food-borne pathogens inoculated into raw meat or poultry products is found to be *Salmonella* spp. (~0.6 kGy) > *Staphylococcus aureus* (~0.5 kGy) > *Listeria* monocytogenes (~0.4 kGy) > *Escherichia coli* O157:H7 (~0.3 kGy) > *Yersinia enterocolitica* (~0.2 kGy) > *Campylobacter* spp. (<0.2 kGy) (Sommers, 2004). The radiation D_{10} values and the order of radiation resistances of the various food-borne pathogens inoculated on to complex ready-to-eat food products such as beef and vegetarian cheeseburgers and frankfurters are shown to be similar to those obtained in raw meat and poultry products. However, the D_{10} values of pathogens decrease three- to fourfold when inoculated on to leafy green vegetables such as iceberg, Boston, green leaf, and red leaf lettuce (Sommers, 2004).

8.2.1.3 Applications of ionizing irradiation

Ionizing radiation can be applied for the reduction of pathogenic microorganisms in food materials, decontamination of food surfaces, extension of the shelf life of certain foods, disinfestation of grains, and elimination of undesirable or toxic materials (CFS, 2009). These applications can also be organized according to the range of delivered dose (Miller, 2005). Low-dose applications (<1 kGy) are concerned with inhibition of sprouting, delaying of maturation, parasite disinfection, and insect disinfestations. Medium-dose applications (1–10 kGy) are used for the control of food-borne pathogens and extension of the shelf life by retarding spoilage. High-dose applications (>10 kGy) are associated with radiation sterilization of foods (Miller, 2005). Kume *et al.*

(2009) investigated the status of food irradiation in the world in 2005. They claimed that the irradiation was usually employed for disinfection of spices and vegetables (which comprise 46% of irradiated foods), disinfestation of grains and fruits (20%), disinfection of meat and fish (8%), and sprout inhibition of garlic and potatoes (22%).

In this section, a few representative examples of recent disinfection applications will be discussed. It has been shown that *Salmonella* contaminates the surface of vegetables and enters into the edible parts of different varieties of plants like lettuce, tomatoes, radish sprouts, bean sprouts, and barley (Tahergorabi *et al.*, 2012). James *et al.* (2010) studied the effect of e-beam on *S. Montevideo* inactivation in tomatoes. They observed significant reductions in the number of microorganisms subjected to >1.5 kGy irradiation. D_{10} values for *S. Montevideo* in tomatoes were 1.07 and 1.5 kGy. In another study, Mahmoud *et al.* (2010) investigated the effects of X-ray at different doses (0.1–2.0 kGy) on *Escherichia coli* O157:H7, *Listeria mnocytogenes*, *Salmonella enterica*, and *Shigella flexneri* inoculated on spinach leaves. They observed more than 5 log cfu/leaf with 2.0 kGy X-ray for all tested pathogens. Mahmoud (2009) also studied the effect of X-ray treatments on inoculated *Escherichia coli* O157:H7, *Salmonella enterica, Shigella flexneri*, and *Vibrio parahaemolyticus* in ready-to-eat shrimp and achieved more than a 6 log cfu reduction of *E. coli O157:H7, S. enterica, S. flexneri*, and *V. parahaemolyticus* with 2.0, 4.0, 3.0, and 3.0 kGy X-ray, respectively. Jia *et al.* (2013) tested the effectiveness of gamma and e-beam irradiation for the inactivation of *Staphylococcus aureus*, *Salmonella enteritidis*, and *Listeria innocua* inoculated on steamed tofu rolls. The results of their study showed that gamma irradiation yielded D_{10} values of 0.20, 0.24, and 0.22 kGY for *S. aureus, S. Enteritidis*, and *L. innocua*, respectively. The respective D_{10} values for e-beam irradiation were 0.31, 0.35, and 0.27 kGy. In a recent study, Kundu *et al.* (2014) determined the radiation sensitivity of *E. coli* O157:H7, non-O157 (VTEC) *E. Coli*, and *Salmonella* strains on beef surfaces to 1 kGy e-beam treatment. *Salmonella* serovars were most resistant to 1 kGy treatment, showing a reduction of ≤1.9 log cfu/g. This treatment reduced the viability of two groups of non-O157 *E. coli* mixtures by ≤4.5 and ≤3.9 log cfu/g. Log reductions of ≤4.0 log cfu/g were observed for *E. coli* O157:H7 cocktails.

8.3 Non-ionizing radiation

Non-ionizing radiation is the term used to describe the part of the electromagnetic spectrum covering two main regions, namely, optical radiation (ultraviolet (UV), visible and infrared (IR)) and electromagnetic fields (EMFs) (extremely low frequency (ELF), microwaves (MWs), and radio frequencies (RFs)). Optical radiation, i.e., another term for light, covers ultraviolet (UV) radiation, visible light, and infrared radiation (IR) regions. Electromagnetic fields (EMFs) arise whenever electrical energy is used. For example, ELF radiation at 60 Hz is produced by power lines, electrical wiring, and electrical equipment. Radio frequencies have wavelengths of between 1 and 100 m and frequencies in the range of 1 to 100 MHz. Microwaves have wavelengths starting from 100th of a meter and have frequencies of about 2.5 GHz (Figure 8.1).

In a radio frequency radiation system the RF generator creates an alternating electric field between two electrodes. The material to be heated is conveyed between the electrodes, causing polar water molecules in the material to continuously reorient to face opposite electrodes. Friction resulting from this molecular movement causes the food material to rapidly heat volumetrically (http://www.radiofrequency.com/rftech.html). The most common commercial RF frequencies are 13, 27, and 40 MHz. RF energy has an ability to penetrate into foods with a depth of more than 5 cm (Ramaswamy and Tang, 2008).

Microwave radiation, which uses electromagnetic energy in the frequency range of 300 to 3000 MHz can be employed successfully to

heat many dielectric materials including foods. The US Federal Communication Commission (FCC) allocates 915 and 2450 MHz bands for microwave heating applications (http://microwaveheating.wsu.edu/factsheet/index.html). Microwaves interact with polar water molecules and charged ions. The friction resulting from molecule alignment and migration of charged ions in a rapidly alternating electromagnetic field generates heat within foods. In other words, the dominant mechanism for dielectric heating is dipolar loss. When a material containing permanent dipoles is subject to a varying electromagnetic field, the dipoles are unable to follow the rapid reversals in the field. As a result of this phase lag, power is dissipated as heat in the material (Bradshaw *et al.*, 1998). Microwaves can penetrate up to 3 cm in packaged foods (Ramaswamy and Tang, 2008).

Infrared (IR) is electromagnetic radiation with longer wavelengths than those of visible light. The wavelength of the peak of the infrared radiation ranges from 780 nm to 1 mm. This range of wavelengths corresponds to a frequency range of approximately 430 THz down to 300 GHz. The infrared range is divided into three regions: short wave or near infrared range (780–1400 nm), medium infrared range (1400–3000 nm), far infrared range (>3000 nm). Near IR is the most used wavelength for industrial heating applications because of the higher temperatures produced (Staack, 2008). However, FIR radiation could be advantageous for food processing because most food components absorb energy in the FIR region (Krishnamurthy *et al.*, 2008a). Infrared energy is emitted or absorbed by molecules when they change their rotational-vibrational movements. Infrared energy causes vibrational modes in a molecule through a change in the dipole moment. When the molecule returns into the normal state, the absorbed energy is transferred into heat. In food products, water is the molecule most affected by IR radiation. Other molecules affected by IR are proteins, lipids, and carbohydrates containing polar groups (-NH, -CO, -OH, C = C) (Staack, 2008).

The wavelength for UV light ranges from 100 to 400 nm. This spectral band can be subdivided into UV-C (180–280 nm), UV-B (280–315 nm), UV-A (315–400 nm), and the vacuum UV range (100–200 nm). Low- and medium-pressure mercury lamps have been the main sources of radiation in most UV-based disinfection systems. The UV-C range is called the germicidal range, since it effectively inactivates bacteria and viruses (Koutchma *et al.*, 2009). Absorption of non-ionizing radiation leads to electronic excitation of atoms and molecules. An atom and most ions consist of electrons orbiting a nucleus of protons and neutrons. When electrons having a unique energy state make a transition from a higher energy to a lower energy, a discrete amount of energy is released as photons of light. Each element emits a unique spectrum of light. UV light is emitted when the difference between energy levels is appropriate (Koutchma *et al.*, 2009).

8.3.1 Impact of non-ionizing radiation on food-borne microorganisms

8.3.1.1 Inactivation mechanism

The microbial destruction in MW and RF radiation is usually attributed to the thermal effect. In other words, microbial inactivation kinetics for these technologies are the same as the inactivation kinetics of conventional thermal processing (IFT, 2000). However, a number of microbiological studies involving MW irradiation showed that the cell death was due to not only heat but also nonthermal (athermal) effects of the microwave electric field. Dreyfuss and Chipley (1980), Singh *et al.* (1994), Banik *et al.* (2002a, 2002b, 2003) tried to demonstrate the existence of nonthermal effects of microwave irradiation. They revealed that "microwaves athermally induced different biological effects by changing the structures by differentially partitioning the ions, altering the rate and/or direction of biochemical reactions" (Banik *et al.*, 2003). The nonthermal effect of RF and MW radiation is attributed to four different mechanisms including selective heating

of microorganisms, electroporation, cell membrane rupture and cell lysis due to electromagnetic energy coupling (Kozempel *et al.*, 1998). However, the studies reporting nonthermal effects have been shown to be inconclusive, and only thermal effects are supposed to exist (IFT, 2000). In fact, Shazman *et al.* (2007) did not detect any athermal (nonthermal) effects due to microwave radiation in a number of chemical, biochemical, and microbiological systems. Additionally, Geveke *et al.* (2002) showed that RF energy at 18 MHz and 0.5 kV/cm does not have any nonthermal effect on *E. coli* K12, *L. innocua*, and yeast in liquids. They claimed that the low-temperature effects of RF were due to heat. It was also indicated that the electric field strength was not enough to cause any rupture in the cell membrane of microorganisms to generate a nonthermal effect.

The infrared power level, temperature of food sample, peak wavelength and bandwidth of IR heating, sample depth, types of microorganisms (spores are more resistant than vegetative cells), moisture content, and the physiological phase of the microorganisms (exponential cells are more sensitive than stationary phase cells) are the important factors for the efficiency of microbial inactivation by IR radiation (Krishnamurthy *et al.*, 2008a). The inactivation mechanisms of microorganisms by IR radiation may include both DNA damage, which is similar to that of UV irradiation, and the thermal effect, which is the same as in MW heating (Hamanaka *et al.*, 2000). Thermal inactivation can damage DNA, RNA, ribosome, the cell envelope, and proteins in microbial cells. However, Sawai *et al.* (1995) demonstrated that RNA, protein, and cell wall damage were more pronounced in *E. coli* cells subjected to far-infrared (FIR) radiation. They claimed that FIR radiation damages RNA polymerase and ribosome in *E. coli*. Though similar changes in the sensitivity are obtained in the case of thermal conductive heating, the pasteurization effect of FIR radiation is much greater than that of conductive heating. Krishnamurthy (2006) also showed cell wall damage, condensation of cytoplasm, and cellular content leakage in infrared heat-treated *S. aureus* cells by using transmission electron microscopy (TEM) and FTIR spectroscopy techniques.

The inactivation mechanism of UV irradiation is related to the absorption of UV photons by DNA or RNA pyrimidine bases. DNA of most living organisms is double stranded, including adenine in one strand and thymine in the other, and, linked by one hydrogen bond, guanine is paired with cytosine by one hydrogen bond (Bank, 1990; Bintsis, 2000; Cadet *et al.*, 2005; Miller *et al.*, 1999; Tornaletti, 2005). The purine and pyrimidine combinations are called base pairs. When UV light of a germicidal wavelength is absorbed by the pyrimidine bases, the hydrogen bond is ruptured (Cieminis *et al.*, 1987; Tornaletti, 2005). New bonds between adjacent nucleotides are structured with the help of high energy of electromagnetic wavelengths found in the UV-C region. This phenomena creates double molecules or dimers (Tornaletti, 2005), shown in Figure 8.3. Dimerization of adjacent pyrimidine molecules is the most common photochemical damage. However, in contrast, cytosine–cytosine, cytosine–thymine, and uracil dimerizations are also identified. Hence, the cell replication is interrupted by formation of numerous dimers in the DNA and RNA of the microbial structure with the effect of other types of damage such as coss-linking of nucleic acids and proteins resulting in cell death (Tornaletti, 2005). In conclusion, the incident light causes a pyrimidine dimer formation on the same DNA strand between two adjacent nucleotides, leading to inhibition of transcription and replication and eventually death of the cell (Bolton and Linden, 2003; Koutchma, 2009).

8.3.1.2 Modeling inactivaton kinetics

The classical approach is commonly used to model thermal microbial inactivation (Chick, 1908). The local change in microbial population (N) is modeled as a first-order decay reaction (similar to Equation (8.1)):

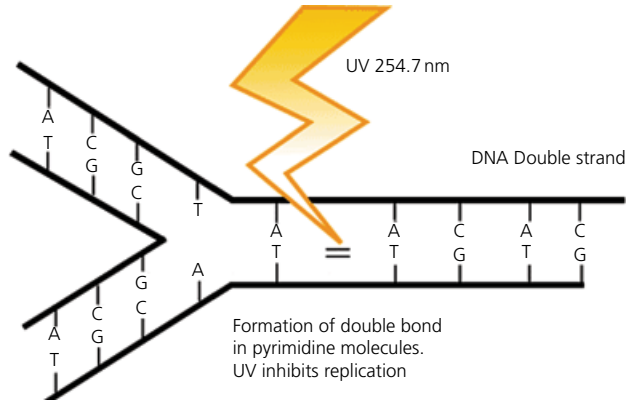

Figure 8.3 Effect of UV-C light on DNA double strand (from http://www.aquafineuv.com/UVTechnology/UVScience.aspx).

$$\frac{dN}{dt} = -kN \tag{8.5}$$

The death of microorganisms can be predicted from a logarithmic form of Equation (8.5) that is known as a log-linear model (Unluturk *et al.*, 2010):

$$\log_{10}\left(\frac{N}{N_0}\right) = -kt \tag{8.6}$$

where N_0 and N are the number of untreated and treated microorganisms (cfu/ml or cfu/cm^2), t is the time, and k (min^{-1}) is the inactivation rate constant. Because k is a function of temperature, it is often described with the Arrhenius equation (Huang *et al.*, 2009).

However, thermal inactivation models for bacteria have shown deviation from first-order kinetics (van Boekel, 2009). Survival curves can have tail and/or shoulder regions (Figure 8.2). These types of non-log-linear sigmoidal shape survival curves are very common in conventional isothermal treatment. It has been shown that a non-log-linear survivor curve can be observed when a mild heat treatment is applied. Geeraerd *et al.* (2000) thoroughly analysed modeling approaches to describe microbial inactivation during mild heat treatment. They formulated structural model requirements and illustrated the different model performances by using a range of experimental data of *Bacillus cereus, Yersinia enterocolitica, Escherichia coli* O157:H7, *Listeria monocytogenes, and Lactobacillus sake.*

However, MW is considered as a non-isothermal process. Valero *et al.* (2014) studied the effect of MW heating on the inactivation of *Salmonella enterica* serover Enteritidis in a potato omelet. They built a log-linear + shoulder inactivation model as a function of MW power levels and considered the effect of temperature during MW heating by developing non-isothermal Weibull models. The log-linear + shoulder model takes into account the resistance period of the microbial population at the beginning of the treatment (Valero *et al.*, 2014). It uses the following equation:

$$N = N_0 e^{-k_{\max} t} \frac{e^{k_{\max} SI}}{1 + \left(e^{k_{\max} SI} - 1\right) e^{-k_{\max} t}} \tag{8.7}$$

where N_0 and N are the initial concentration (cfu/g) and the cell concentration (cfu/ml), respectively, after a treatment time t (s), $k_{\max}$ is the maximum inactivation rate (s^{-1}) and SI is the shoulder length (s).

The Weibull distribution is defined as a simple kinetic model used to describe microbial inactivation by thermal and nonthermal treatment methods. It is a simple model and usually used to describe the concavity or convexity of non-linear survival curves as a function

of inactivation time. This model has been well described by other authors (e.g., Bialka *et al.*, 2008; Buzrul and Alpas, 2007; Buzrul *et al.*, 2005; Van Boekel, 2002; Peleg and Normand, 2004) and uses the following equation:

$$\log N(t) = -bt^n \tag{8.8}$$

where $N(t)$ is the instantaneous survival ratio and b and n are the temperature dependent rate and shape parameters, respectively. The non-isothermal Weibull model is used to describe the effect of temperature on the inactivation rate of *Salmonella enterica* in a potato omelet (Valero *et al.*, 2014). The non-isothermal model is developed using the Weibull model and the model developed by Mattick *et al.* (2001). Thus the following survival rate equation is obtained:

$$\frac{d\log N(t)}{dt} = -b\left[T(t)\right]n\left[N(t)\right]\left\{-\frac{\log N(t)}{b\left[T(t)\right]}\right\}^{\left(n\left[T(t)\right]-1\right)/n\left[T(t)\right]} \tag{8.9}$$

Bennloch-Tinoco *et al.* (2014) investigated the inactivation of *Listeria monocytogenes* in a kiwifruit puree by conventional and microwave heating. They showed that the survival behavior of *Listeria* monocytogenes under MW processing was properly fitted to first-order kinetics.

First-order kinetics (Equation (8.5)) and exponential reduction is observed in the population of vegetative cells when treated with infrared radiation. However, shoulder and tailing effects are reported for bacterial spores exposed to IR heating. For example, Hamanaka *et al.* (2000) and Daisuke *et al.* (2001) proclaimed shoulder and tailing effects for *Bacillus subtilis* spores exposed to infrared heating. Sawai *et al.* (2003, 2006) indicated that inactivation of *Escherichia coli* due to both IR heating and thermal conductive heating followed first-order reaction kinetics (Equations (8.5) and (8.6)).

The inactivation curve of microorganisms exposed to UV irradiation has a sigmoidal shape that is composed of an initial plateau region, linear part, and tailing phase. The behavior of the survival curve is explained by a multihit phenomena, i.e., inactivation efficiency decreases by the effect of the multiple hit. In this phenomenon, multiple UV contacts a single cell or a single UV light affects a group of cells. Thus, less-resistant cells are eliminated first, and then more resistant cells are left to form a tail in the survival curve. When there is a resistivity to inactivation of the target microorganism at the beginning of UV irradiation, this behavior is represented as a "shoulder" effect in the non-linear inactivation curve (Figure 8.3) (Benabbou *et al.*, 2007; Bermudez-Aguirre *et al.*, 2009; Marugán *et al.*, 2008). If the inactivation rate slows down at the end of the inactivation process, this part of the inactivation curve is named the "tailing" region (Bialka *et al.*, 2008; Buzrul and Alpas, 2007; Fernández *et al.*, 2007; Marugán *et al.*, 2008; Van Boekel, 2002). Tailing of inactivation curve occurs by the shadowing effect of high concentration of non-microbial solid particles or improper mixing of liquid food (Marugán *et al.*, 2008). Another reason for the tailing effect might be the increase in resistance and the survival rate of a microbial population in disinfected liquid food. Also, agglomeration of the death population is likely to prevent UV penetration to living cells due to improper mixing.

The survival curve of microorganisms can be described by using both linear and non-linear models. Besides the log-linear model (Equation (8.5)), the non-linear behavior of microbial inactivation is described by using some empirical models (e.g., the Weibull model (Equation (8.8)), the modified Chick–Watson model, the Hom model, etc.) (Atilgan, 2013). The Weibull model is explained in previous paragraphs of this section.

The Chick–Watson equation is an empirical disinfection model considering the inactivation rate of a target microorganism changing as an exponential function of the disinfectant agent concentration (Chick, 1908; Watson, 1908). Integrated as a function of time, the model can be represented as

$$\left(\frac{N}{N_0}\right) = \exp\left(-kC^n\ t\right) \tag{8.10}$$

where k represents the Chick–Watson coefficient of specific lethality (min^{-1}), C is the concentration of the disinfectant (mg/l), n is the dilution coefficient, and t is the disinfection time (min). At low concentrations of microbial population exposed to a long treatment period, the rate of inactivation stays constant until the end of the inactivation process (Huang *et al.*, 2009; Marugán *et al.*, 2008). In order to describe the outward convexity or inward concavity in the inactivation curve, the Chick–Watson model could be modified by introducing two parameters in Equation (8.10) (Cho *et al.*, 2003; Marugán *et al.*, 2008):

$$\log\frac{N}{N_0} = -k_1\left[1-\exp\left(-k_2 t\right)\right] \tag{8.11}$$

where k_1 is the inactivation rate constant (min^{-1}) for the log-linear inactivation part of the curve and k_2 is the first-order UV decay constant (min^{-1}), representing the tailing effect of the decelerated inactivation.

The Hom model incorporates the parameter h. The expresion is

$$\log\left(\frac{N}{N_0}\right) = -k't^h \tag{8.12}$$

where k' is the inactivation rate constant of the Hom model (min^{-1}) and h represents the UV penetration rate constant that describes the tailing or shoulder effect of the inactivation rate. When $h=1$, this equation simplifies to the log-linear equation. For the case $h<1$, the equation permits the fitting of the tailing (inward concavity) part of the inactivation curve, while for $h>1$, the shoulder (outward convexity) part is predicted (Haas and Joffe, 1994; Lee and Nam, 2002; Marugán *et al.*, 2008). A small k′ value (inactivation rate constant) indicates that the microorganism shows a larger resistivity to treatment.

8.3.1.3 Applications of non-ionizing irradiation

There have been a number of reviews and studies in the area of MW and RF radiation, which include publications by Chandrasekaran *et al.* (2013), Banik *et al.* (2003), Ramaswamy and Tang (2008), Marra *et al.* (2009), Piyasena *et al.* (2003), and Wang *et al.* (2003) who considered the major technological aspects and applications of MW and RF treatment in food processing.

Radio frequency has been applied for cooking, heating, drying, post-baking, thawing of frozen food products, and has been used in meat processing (Piyasena *et al.*, 2003; Marra *et al.*, 2009). For example, Byrne *et al.* (2010) performed a study to investigate the effect of RF radiation on the inactivation of *Bacillus cereus* and *Clostridium perfiringens* vegetative cells and spore cocktails in pork luncheon meat. The results of their study showed a reduction in *B. cereus* vegetative cells and spores of 5.4 and 1.8 $\log_{10}$ cfu/g, respectively, while the corresponding reduction for *C. perfringens* vegetative cells and spores were 6.8 and 4.1 $\log_{10}$ cfu/g, respectively.

RF radiation has been also considered as an alternative method for the pasteurization of liquid foods (Piyasena and Dussault, 2003; Awuah *et al.*, 2002, 2005). Geveke and Brunkhorst (2004, 2008) and Geveke *et al.* (2007) assessed the effect of RF treatment on microbial inactivation in a number of different beverages. They showed that RF treatment has a potential in inactivation of *E coli* K12 by up to 3, 3.3, and 4.8 log cfu/ml in apple juice, orange juice, and apple cider, respectively. More recently, Jeong and Kang (2014) investigated the effect of RF heating on the inactivation of foodborne pathogens (*Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium) in red and black pepper. They reported more than 7 log cfu/g reduction in the number of *E. coli* O157:H7 and *S.* Typhimurium under studied conditions by means of an RF system with 27.12 MHz. The authors proposed to use RF heating to control pathogens and reduce mositure levels in spices.

MW heating can be used for cooking, blanching, drying, pasteurization, and sterilization of food materials and MW radiation can be easily combined with other conventional methods. That is why it has vast applications in the field of food processing. These applications have been extensively reviewed by Chandrasekaran *et al.* (2013) and Salazar-Gonzalez *et al.* (2012). In a recent study, microwave pasteurization was used for the inactivation of *Salmonella* Typhimurium in the yolk of shell eggs (Shenga *et al.*, 2010). In another study, De La Vega-Miranda *et al.* (2012) demonstrated that water-assisted microwave treatment has an effect against *Salmonella* Typhimurium which resulted in the reduction of 4–5 log cycles of microbial population in fresh jalapeno peppers and coriander foliage. Guiliani *et al.* (2010) applied microwave processing to a cream of asparagus and achieved a twofold reduction in the number of *Alicyclobacillus acidoterrestris.* More recently, the US Food and Drug Administration has approved the microwave sterilization process for mashed potatoes in trays and salmon fillet in sauce in pouches (Brody, 2012).

Infrared radiation has been applied in drying, baking, roasting, blanching, pasteurization, and sterilization of food products. It can be employed for inactivation of bacteria, spores, yeast, and mold in both liquid and solid foods (Krishnamurthy *et al.*, 2008a). The studies pertinent to pathogen inactivation by IR radiation in different types of food materials such as liquid, solid, and non-food materials is summarized by Krishnamurthy *et al.* (2008a, 2009) and Rastogi (2012). Krishnamurthy *et al.* (2008b) demonstrated the potential of IR heating in milk processing for effective inactivation of *Staphylococcus aureus*. Depending upon the treatment conditions, the population was reduced from 0.10 to 8.41 $\log_{10}$ cfu/ml. Erdogdu and Ekiz (2011, 2013) applied far infrared (FIR) and UV-C radiation for the surface pasteurization of black pepper seeds and cumin seeds. They reported complete elimination of the microorganisms (total mold and yeast, *E. coli, and B. cereus*) in the black pepper seed samples after 4.7 and 3.5 min FIR treatment at 300 and 350 °C. UVC treatment after FIR application did not exhibit any significant additional reduction in the total mesophilic aerobic bacteria (TMAB) count. However, when FIR is combined with UV, it is stated that TMAB of the cumin seeds is decreased to the target level of 10^4 cfu/g after 1.57, 2.8, and 4.8 min FIR treatment at 300, 250, and 200 °C, respectively, following a 2 h UVC treatment. More recently, Huang and Sites (2012) achieved maximum of 1.6 log reductions in the number of *Listeria* monocytogenes inoculated on the surface of ready-to-eat (RTE) chicken meats by near infrared (NIR). They found the NIR surface pasteurization process more effective than the hot water immersion process to eliminate *Listeria* monocytogenes.

UV-C irradiation between 200 and 280 nm has been widely used for disinfection of transparent, semi-transparent liquid foods in addition to solid food surfaces due to its germicidal effect (Bolton and Linden, 2003; Koutchma *et al.*, 2009). The Food and Drug Administration (FDA) has allowed the use of ultraviolet irradiation to eliminate food-borne pathogens and other microorganisms in juice products since November 29, 2000. Unluturk *et al.* (2004) studied the effects of UV light efficiency on the destruction of *Escherichia coli* K-12 bacteria in the model fluids using laminar and turbulent flow UV systems in terms of the single factor, i.e., UV absorbance. Ngadi *et al.* (2003) evaluated the effect of pH, depth of food medium, and UV dose on *E.coli* O157:H7 reduction in egg white and apple juice exposed to UV for 0–16 minutes at 0.315 mW/cm^2. There are also many studies recently published and cited in the literature about the efficacy of UV-C light for the reduction of different microorganisms by UV irradiation (Franz *et al.*, 2009; Char *et al.*, 2010; Unluturk *et al.*, 2010; Oteiza *et al.*, 2010; Ukuku and Geveke, 2010; Choudhary *et al.*, 2011; Bandla *et al.*, 2012; Gabriel, 2012; Geveke and Torres, 2012; Ochoa-Velasco and Guerrero-Beltrán, 2012).

In summary, the UV disinfection method is mainly applied in the treatment of spent process water in the food and beverage industries (Chmiel *et al.*, 2002; Hassen *et al.*, 2000), treatment of drinking water (Lehtola *et al.*, 2004), pasteurization of milk and clarified fruit juices (Bandla *et al.*, 2012; Choudhary *et al.*, 2011; Koutchma *et al.*, 2004; Matak *et al.*, 2005; Geveke and Torres, 2012). It has also been applied in tropical fruit juice production such as pitaya, mango, guava and pineapple, and mango and pineapple juices (Keyser *et al.*, 2008; Ochoa-Velasco and Guerrero-Beltrán, 2012), liquid egg products (Unluturk *et al.*, 2008, 2010), and grape juice processing (Unluturk and Atilgan, 2014; Guerrero-Beltrán *et al.*, 2009). UV-C light treatment has also been used in the food industry for different purposes, including air sanitation in meat and vegetable processing, and reduction of pathogen microorganisms in red meat, poultry, and fish processing (Liltved and Landfald, 2000; Wong *et al.*, 1998).

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