EFFECT OF ELECTRON BEAM IRRADIATION AND SUGAR CONTENT ON

KINETICS OF MICROBIAL SURVIVAL

A Thesis

by

OSCAR RODRIGUEZ GONZALEZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Biological and Agricultural Engineering

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Approved by:

Chair of Committee,   Elena Castell-Perez
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ABSTRACT

Effect of Electron Beam Irradiation and Sugar Content on Kinetics of Microbial Survival.

(August 2005)

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The killing effectiveness of electron beam irradiation has not been completely characterized. The type of microorganisms and the composition of food have a direct effect on the efficiency of this technology. The objectives of this study were to select a surrogate suitable for use in electron beam irradiation studies of fruits and to evaluate the effect of sugar content on the kinetics of microbial damage and recovery. A 2.0 MeV Van de Graaff linear accelerator was used to apply irradiation (up to 5.0 kGy), using different configurations, on gelatin-based systems with the addition of sugars. The systems were inoculated with pathogenic and non-pathogenic bacteria strains (surrogates). Initial studies showed that *Escherichia coli* K-12 MG1655 is a suitable surrogate that represents the damage induced to common fruit pathogens by irradiation. The reduction in bacteria population can be maintained by storing samples at 4°C. An increase in temperature up to 20°C was enough for the damaged population to recover in 48 hours. Gelatin-based systems proved to be a simple and inexpensive medium to evaluate the effects of irradiation (up to 5.0 kGy) on selected bacteria. Reduction of the system dimensions and their positioning related to the beam source were key factors in increasing the killing effectiveness of irradiation. The sugar levels (up to 8 %) used to mimic the maturity of cantaloupes had no effect on the radiation D_{10} values and the recovery of the surrogate

population quantified as Generation Times. The resistance of the surrogate to irradiation was validated in an optimum configuration and in cantaloupes. Temperature and sugar content caused significantly higher changes to the physical structure of the gel-based systems than irradiation (1.0 kGy). Plate counts and light microscopy techniques demonstrated that the structure of the gelatin-based systems allow for motility of the bacteria in a 3-D array (length, width and depth). When little information was available about the effectiveness of using a low energy linear accelerator, the inoculation of gelatin-based systems proved to be a reliable method to select a suitable surrogate and to predict the effects of irradiation on bacteria as a function of sugar content.

DEDICATION

To God,

who directed my family and friends to help me to persevere in my second thesis.

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Thanks to the Food Safety Engineering group for their help.

NOMENCLATURE

A — Inoculum, $\log_{10}$ CFU/ml.

$\overline{A}$ — Area of movement, μm^2.

B — Population, $\log_{10}$ CFU/ml at the beginning of the exponential phase.

C — Population at the end of the exponential phase, $\log_{10}$ CFU/ml.

CV — Coefficient of variability of the mean, %.

d — Displacement, μm.

D — Decimal reduction time, or time required for a logarithmic cycle reduction in the microbial population, hours.

D — Dose, kGy.

D_0 — The negative reciprocal of the slope of the curve of S/S_0 and D, kGy.

D_{10} — Radiation dose required to decrease 1 $\log_{10}$ CFU/ml, kGy.

D_n — Displacement towards the axis (x or y), μm.

$\overline{D}$ — Average diameter, μm.

E — Elastic or Young's Modulus, kPa.

ε_{fr} — Strain at fracture, %.

GT — Generation time, hours^{-1}.

I — Population injured by a treatment at a given dose, $\log_{10}$ CFU/ml.

K — Concentration constant, %.

k — First order reaction rate constant, hours^{-1} or kGy^{-1} in irradiation treatments.

Ki — Population killed by a treatment at a given dose, $\log_{10}$ CFU/ml.

L — Lag phase, hours.

m — Number of generations (number of times the cell population doubles during the time interval).

N Microbial population, at any time (t) in hours or dose (D) in kGy, CFU/ml.

N_o Initial microbial population, CFU/ml.

n Number of generations (number of times the cell population doubles during the time interval).

O Survival population recovered in media with optimal conditions, $\log_{10}$ CFU/ml.

P Population of bacteria, %.

R Population recovered in an optimum medium, $\log_{10}$ CFU/ml.

R^2 Linear regression coefficient, dimensionless.

r Population recovered in a restricted medium, $\log_{10}$ CFU/ml.

RF Recovery factor, dimensionless.

ρ_b Bacteria density, bacteria/100 μm^2.

S Number of surviving cells at dose D, $\log_{10}$ CFU/ml.

$[S]$ Concentration of limiting food, %.

S_0 Original number of irradiated cells, $\log_{10}$ CFU/ml.

$[S_t]$ Total concentration of a macronutrient, %

Su Survival population recovered in media under sub-optimal conditions, $\log_{10}$ CFU/ml.

S_w Swimmers, %.

σ_{fr} Strength at fracture, kPa.

T Growth rate, number of cellular divisions per unit time, $\log_{10}$ CFUml^{-1}/h.

T_{max} Maximum growth rate, $\log_{10}$ CFUml^{-1}/h.

t Time, hours.

t_A Time when a microbial population begins the exponential phase, hours.

t_B Time when a microbial population reaches the stationary phase, hours.

t_d Doubling time or generation time (GT), hours.

t_m Time of measurement, s.

v Velocity, μm/s.

x_n Horizontal position relative to the origin, μm.

y_n Vertical position relative to the origin, μm.

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CHAPTER I

INTRODUCTION

Sickness from ingestion of food-borne pathogens is a serious cause of lost wages and medical expense in the United States and around the world. Fruits and vegetables can become contaminated with microorganisms capable of causing human diseases while the plants are still on the field, during harvesting, transport, processing, distribution, and marketing. The United States Department of Agriculture (USDA), the Food and Drug Administration (FDA), the World Health Organization (WHO), industries, and retailers have a strong interest on the evaluation of alternatives to minimize this problem. One approach is to develop reliable decontamination strategies using technologies such as irradiation.

A major benefit of irradiation technology is its effectiveness as a tool in eliminating pathogens in foods. Electron beam irradiation is a promising technique for treatment of food products because it causes minimal changes on some product quality attributes while ensuring its safety. Within the approved dosages, the technology has been shown to destroy at least 99.9 percent of common food-borne pathogens such as *Salmonella*, *Campylobacter jejuni*, *Escherichia coli*, and *Listeria monocytogenes* associated with meat and poultry (GAO, 2000). Low (up to 1.0 kGy) to medium (1.0-10.0 kGy) irradiation doses can produce the same decontamination effects to food-borne

This thesis follows the style of the *Journal of Food Engineering*.

bacteria, delaying spoilage of highly perishable foods, and extending the shelf-life of fruits (Crawford and Ruff, 1996). Irradiation treatments cannot induce radioactivity in the product, are safe for the consumer, and do not affect the environment (ICGFI, 1991). Although the effects of electron beam irradiation on the quality of a wide variety of fresh fruits are still under investigation, additional research is needed to establish appropriate treatment plans for specific food items.

In the study and application of irradiation, like in all preservation processes, the use of consistent parameters to describe the inactivation of pathogens should improve the efficiency of future investigations and encourage uniformity in the methodologies for establishment of minimum process requirements (FDA/CFSAN, 2000). Surrogate microorganisms (usually bacteria, but sometimes yeast and molds) are used in process-validation studies in situations where one would not choose to use a pertinent target pathogen; such as in a pilot plant, at the bench, in a piece of equipment or the production environment where assessing the efficacy of various new or experimental strategies with a high level of pathogens can compromise the safety of the workers or the integrity of the processing environment (Slade, 2003). Therefore, the use of surrogates is a reliable tool to determine the effectiveness of the treatment, for which non-pathogenic strains need to be identified and their significance evaluated (FDA/CFSAN, 2000).

Consumers have long valued fruits for their flavor, appearance and texture. These desirable fruit attributes are due to the presence of sugar, for example, (1) flavor is fundamentally the balance between sugar and acids, and specific flavor constituents are often glycosides; (2) the color of fruits is sometimes due to sugar derivatives of

anthocyanidins; (3) texture is governed by structural polysaccharides and; (4) ascorbic acid (vitamin C) is commonly considered to be a sugar derivative found widely in fruits (Whiting, 1970). The level of ripening and fruit quality in fruits such as melons is assessed by their sugar content. Therefore the study of the accumulation of sugars during fruit development is important (Villanueva et al. 2004).

The common approach is to develop a model food system with controlled amounts of a specific component (i.e. sugar). The simplest method is to combine one ingredient with water such as a simple starch-water system; however, this method is limited because a direct extrapolation to real products (i.e. fruits) is practically impossible (Wischmann et al., 2002). More complex systems containing formulations similar to real food products are necessary in order to fully understand the functionality of the ingredient under investigation, for which a myriad of food models have been developed to investigate different properties (i.e. physical and chemical), and to determine the influence of process conditions on the product (Wischmann et al., 2002).

Few studies have been conducted on model food systems to determine the kinetics of growth, injury, and survival of microbial pathogens and surrogates under low dose electron beam irradiation. Furthermore, the growth characteristics of the main three food pathogens (*E. coli*, *L. monocytogenes* and *Salmonella*) under irradiation treatments have not yet been established.

Based in past experiments the actual regulations establish a maximum dose of 1.0 kGy to be applied to fresh foods, but this value may vary depending on the commodity and the pathogen under treatment. Therefore dose limitations must be evaluated in

parallel to quality changes to reduce the population of pathogens.

The main goal of this study was to quantify the effects of electron beam irradiation parameters (dose) and fruit maturity stage (i.e. sugar content) on the death and probability of recovery of selected surrogates. This goal was achieved by accomplishing the following specific objectives:

1.   Evaluation of the effect of fruit composition, maturity stage (sugar content) and environmental conditions (temperature) on the effectiveness of electron beam irradiation as a decontamination technology using model food systems.

2.   Establishment of the kinetics of the death and recovery of the selected surrogate in different media compositions as a tool for prediction of the effect of electron beam irradiation on common pathogens.

CHAPTER II

LITERATURE REVIEW

2.1 The problem of fruit safety

The U. S. Food and Drug Administration (FDA) has reported that 54% of the fruit production in the U. S. is commonly utilized as fresh and 46% as processed, with a value of 4.4 billion dollars in average for non-citrus fruits. In 1999, the FDA conducted a survey on the microbial contamination of high volume of imported fresh produce; the survey showed that 4% of the samples were contaminated (FDA/CFSAN, 2001a). The Center for Disease Control (CDC, 2004) reported 66 outbreaks, 12,357 cases and 2 deaths due to food-borne pathogens present in fresh fruits in the U. S. from 1993 to 1997 (Olsen et al., 2000). Although the incidence of food-borne illnesses is low, during the last several years this proportion has increased drastically. Because most fresh produce is likely to be consumed raw, without undergoing processes, this represents a serious risk to consumer health.

The increased incidence of food-borne illnesses and the consumer desire for minimally processed safe foods poses a challenge to the food industry and its regulators (Ross and McKeein, 2002).

2.2 Pathogens of importance to fruit safety

Results from the CDC annual surveillance in 2003 reported that from a total of 15,600 laboratory-diagnosed cases of infections, 6,017 were caused by *Salmonella*; 443

by *Escherichia coli* O157:H7; and 138 by *Listeria* species (CDC, 2004). These results, compared with the diseases occurring since 1996, show that the 2003 incidence of infection was lower than the average annual incidence for 1996-1998. Those infections from *E. coli* O157:H7 decreased by forty two percent; those from *Salmonella* decreased seventeen percent; and those from *Listeria* did not continue to decline in 2003, as observed during the preceding 4 years. Of nine pathogens, *Salmonella* was the leading cause of illness; *E. coli* took the fifth place and *Listeria* the seventh. The Center for Science and Public Interest (CSPI, 2004) reported that from all the outbreaks reported related to food-borne illnesses between 1990 and 2003, twelve percent were related to fresh produce, from which ten percent were due to contamination after leaving the farm (handling). These results suggest that a decontamination treatment is needed before the products reach the consumer.

It is necessary to clearly differentiate between the most resistant species and the commonly cause of illnesses due to consumption of contaminated foods. In general, gram positive bacteria are more resistant than gram negative bacteria to environmental stresses, but a variety of responses have been demonstrated to affect the resistance, growth and survival during food storage of the various genera and species of pathogens (Johnson, 2003).

2.2.1 Escherichia coli *species*

E. coli, which is classified as gram negative, with non-sporing rods, often motile, with peritrichate flagella, easy to cultivate, aerobic, and facultative anaerobic, is considered by microbiologists as an indicator of feces contamination in water sources

and milk (Bell and Kyriakides, 2002a). Different types of pathogenicity are conferred to certain strains, depending on the virulence genes acquired: entero-pathogenic (EPEC), entero-toxigenic (ETEC) entero-haemorrhagic (EHEC), and entero-aggregative (EAEC). The different virulent factors expressed by the organism (colonization, ability to invade epithelial cells of the small intestine, haemolysin production and toxin production), lead to the different strains of *E. coli* associated with a wide variety of types of disease. From this variety the ones that produce "shiga" toxins (EHEC) are more important for the food industry to ensure highest safety standards due to their very low infective dose (particularly *E. coli* O157:H7), and are called Vero Citotoxigenic or VETC (Bell and Kyriakides, 2002a).

Surveys state that an increasing number of outbreaks of VETC *E. coli* have implicated fruit as a primary vehicle, where in most of the cases the source of the contamination has been animal feces in the field. The high levels of pathogens remaining viable in the soil (10^5-10^{10} colony forming units) are subject to a variety of factors including exposure to sunlight and drying, and once introduced in fruits or vegetables they can survive for extended periods (Bell and Kyriakides, 2002a).

Outbreaks where contamination with *E. coli* O157:H7 was identified in fresh fruits around the world are reported by the FDA, seven cases in un-pasteurized apple juice, one in melons, and one in fruit salads (FDA/CFSAN, 2001b).

2.2.2 Listeria *species*

Currently, six clearly distinguishable species are recognized: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. grayi*, as being the cause of

listeriosis illnesses identified since the 1980's; the first two are the most commonly distributed in the environment (Bell and Kyriakides, 2002c). *Listeria* species are classified as gram positive, short, non-sporing rods, catalase positive and facultatively anaerobic (ICMSF, 1996). *L. monocytogenes*, the main human pathogen transmitted by contact with animals, cross-infection in hospitals and food-borne infection, is found in fruits and vegetables that can be contaminated from soil and water sources. High levels ($>10^3$ cells) are usually required to cause infection and illness and very low levels that may be present are not considered to represent a significant hazard (Bell and Kyriakides, 2002c).

Due to their importance in foods, several studies have addressed the stress responses and resistance properties of *Listeria* species in food systems. In general, acid-adapted *Listeria* species are more resistant at low pH (3.5 to 4.0) conditions (Johnson, 2003). Although a few cases relating *Listeria* species have been documented, their detection in products with similar composition as cabbage, coleslaw, carrots, asparagus and broccoli suggests a potential presence in fresh fruits (FDA/CFSAN, 2001b).

2.2.3 Salmonella *species*

These species has been recognized for over 100 years as the cause of illnesses from mild to severe poisoning (gastroenteritis), severe typhoid (enteric fever), paratyphoid, bacteraemia, septicaemia, and a variety of associated long-term conditions. *Salmonellae* are classified as facultative anaerobic, gram negative, and straight small (0.7-1.5 x 2.0-5.0μm) rods which are usually peritrichous flagella (Bell and Kyriakides, 2002b). The infective dose of *Salmonella* species has been considered to be in excess of

10^5 cells, but a number of outbreaks has been recorded in products where the infective dose was <10-100 cells; they can be present in large numbers in animal wastes, and can survive for periods up to 41 weeks in stored slurry, which suggests that one of the main sources of contamination of fruits is the presence of animal waste in the field (Bell and Kyriakides, 2002b).

Fresh fruits are implicated less frequently to contamination with *Salmonella* species, and most of the cases of *salmonellosis* have been attributed to melons (10), un-pasteurized apple juice (3), citrus juices (2), and berries (1) (FDA/CFSAN, 2001b).

A complete review of different studies on the responses and adaptation of *Salmonella* species to different stresses (sugars, acids, water activity) in media and food environments was made by Johnson (2003). The variability in results of all the studies suggests that both the composition of the food matrix and the strain are important when analyzing stress responses, for which it is necessary to develop appropriate biomarkers to demonstrate the expression of a stress response and to distinguish stressed from injured cells. It is necessary to use standard strains to accurately compare results from different laboratories (Johnson, 2003).

2.3 Electron beam irradiation as a decontamination technology

In the United States, an estimated 97 million pounds (44.1 million kg) of food products are irradiated annually, from which fruits and vegetables account for about 1.5 million pounds (0.7 million kg or 0.002%) of the total annual consumption (GAO, 2000).

2.3.1 Food irradiation and regulations

The beginning of the story of ionizing radiation in modern physics began with the unexpected discovery of X-rays by Roengten on November 8, 1895. From this date until the present different events were important in the development of the application of this technology to foods as listed in Table 2.1 (Turner, 1995).

In 1998, the World Health Organization (WHO) recommended that decay of indigenous microflora and post-handling contaminants can be eliminated or delayed by radiation dose levels that do not adversely affect the sensory qualities of many fruits and vegetables. It also states that fecal matter or water containing feces should never come into contact with fruits and vegetables, as even the most powerful treatment (irradiation) cannot be relied on to eliminate some of the pathogens they may contain (Beuchat, 1998). The WHO concluded that, although irradiation cannot be used for inactivation of viruses, it could be an extremely effective tool in reducing populations of pathogenic microorganisms from the surface of raw fruits and vegetables, and its application on a large scale would appear to have an exceptional merit; however, there is a need to evaluate the tolerance of most fruits and vegetables to the radiation doses required for controlling various pathogenic microorganisms (Beuchat, 1998).

The American Society for Testing of Materials (ASTM) is the world's largest source of voluntary consensus standards, where individual standards are developed by tasks groups (Farrar et al, 1993). One group has been developing standards on how to use gamma, electron beam, and X-ray facilities for radiation processing, and standards

Table 2.1

Important events in the development and application of irradiation as a sanitizing method of fruits and vegetables

Year	Event
1895	Discovery of X rays by Roentgen.
1896	Becquerel discovers radioactivity, and proposes its use to destroy microorganisms.
1905	Scientists receive patents for a food preservative process that uses ionizing radiation to kill bacteria in food.
1953-1980	The U. S. Government forms the National Food Irradiation Program. Under this program, the U. S. Army and the Atomic Energy Commission sponsor many research projects on food irradiation.
1958	The Food, Drug, and Cosmetic Act is amended and defines sources of radiation intended for use in processing food as a new food additive. Act administered by FDA.
1963	The FDA approves the use of irradiation in fruits and vegetables.
1964-1968	The U. S. Army and the Atomic Energy Commission petition to FDA approve the irradiation of several packaging materials.
1971	FDA approves the irradiation of several packaging materials based on the 1964-48 petition.
1980	USDA inherits the U. S. Army's food irradiation program.
1986	FDA approves irradiation at specific doses to delay maturation, inhibit growth, and disinfect foods, including vegetables and spices.
1992	The World Health Organization endorses the use of food irradiation as a "perfectly sound food preservation technology".
Present	More than 40 countries have approved the use of irradiation in foods.

Adapted from GAO (2000), Turner (1995), and NEI (2004).

on how to treat dose uncertainties. These standards are being coordinated with other organizations as the International Organization of Legal Metrology (OIML), the Association for Advancement of Medical Instrumentation (AAMI), the European Committee for Standardization (CEN) and the International Consultative Group on Food Irradiation (ICGFI).

2.3.2 Sources of irradiation

2.3.2.1 Gamma rays

Gamma rays are produced by radioactive isotopes such as Cobalt-60 and Cesium-137, and are produced in the form of metal "pencils", and as such, offers much in the way of both convenience and safety (Radomyski et al., 1994).

Although no longer used, Cesium-137 was proposed as a radiation source in the early 1970s, and was based on the availability of vast quantities of unprocessed and encapsulated Cesium-137 from U. S. Government's stock of byproducts from nuclear energy and nuclear weapon production programs. Cobalt-60 is an isotope of Cobalt (Co), which occurs naturally as a non-radioactive metal, and is mined from ore deposits. The gamma rays emitted are photons with very short wavelengths, similar to ultraviolet light and microwaves but with much higher energies and are used in cancer therapy and in diverse industrial processes (Brescia, 2002).

2.3.2.2 Electron beams

Electron beams (E-beams) are produced by Van de Graff generators or linear

accelerators (LINAC), which are powered by electricity. Electron beam facilities, initially built to irradiate medical equipment, are being used for food treatment, and consist of a conveyor or cart system where the product to be irradiated moves through the electron beam at a predetermined speed to obtain the desired dosage (Brescia, 2002).

X-rays are electromagnetic radiation produced when energetic electrons hit a target, and are emitted by a heated cathode whose potential may be the order of 30 to 50kV above the target (made of a material such as tungsten or molybdenum).

The origins of both electron beams and X-rays can be traced to 1895 (Table 1), when a paper published by Roentgen described the production of X-rays, and to 1948 when results of experiments on 22 species of bacteria with electrons and X-rays prompted an interest from medical products manufacturers who developed the first commercial sterilizer, a small Van de Graaff accelerator (Brescia, 2002). Regardless of the source, the effect of ionizing energy on food is identical. Energy penetrates the food and its packaging but with X-rays and gamma-rays most of the energy simply passes through food without leaving residues. Most of the energy that does not pass through the food is converted into heat while some of the energy produces DNA strand breaks that inactivate bacteria.

E-beam accelerators work on the same principle as a television tube, where electrons are emitted from a cathode and accelerated by an electric or magnetic field in a vacuum, and instead of being widely dispersed and hitting a phosphorescent screen at low energy levels, the electrons are concentrated and accelerated to a higher energy (approximately 99% light speed) and finally pass through a thin metal foil and enter air

at normal pressure (Brescia, 2002).

Although electrons are less penetrating than gamma rays, they can be very useful for irradiating large volumes of small food items. Since electrons can be converted to X rays, which are slightly more penetrating than gamma rays produced from Cobalt-60, linear accelerators are versatile alternatives to isotope sources (Radomyski et al., 1994). Other differences include lower penetration power, switch on/ switch off capability, high efficiency, high throughput, and superiority to gamma rays in terms of dose rate (Brescia, 2002). Cost estimates show that irradiation is competitive and often more economical than other residue-free quarantine treatments (ICGFI, 1991).

Irradiation efficiency depends on the irradiation source, its characteristics and the irradiation technique, as well as the type of material, its geometric dimensions, its shape and the packaging material (Brescia, 2002).

2.3.3 Approved doses for irradiation of foods

The cumulative evidence from over four decades of research indicates that irradiated food is safe to eat, is not radioactive, it does not contain toxic substances resulting from the treatment, does not produce more virulent pathogens, and nutritional losses are similar to other form of food processing when applied at the approved doses (GAO, 2000). A list of the approved food products for irradiation and the dosage permitted in the U. S. is presented in Table 2.2.

2.3.4 Dose measurement and calculation

The primary physical quantity used in dosimetry is the absorbed dose, and it is

defined as the energy absorbed per unit mass from any kind of ionizing radiation in any target. The unit dose commonly used is the Gray (Gy) or Joules per kilogram. The older unit radian (rad) is defined as 100 erg/g, and 1.0 Gy is equivalent to 100 rads (Turner, 1995). The dose distribution in a food product is the variation in the absorbed dose throughout the product between the minimum (D_{min}) and the maximum (D_{max}) absorbed dose. Dose uniformity is defined as the ratio D_{min}/D_{max} (Wilkinson and Gould, 1996).

2.3.4.1 Types of dosimeters

Dosimetry is essential for quantifying the incidence of various biological changes, as a function of the amount of radiation received (dose-effect relationship), and for comparing different experiments (Turner, 1995). Twenty-four (24) dosimetry standards have now been published as ISO/ASTM standards, and two of the standards are specifically for food irradiation applications. The majority of the standards apply to all forms of gamma, x-ray, and electron beam radiation processing, including dosimetry for the sterilization of health care products; for the radiation processing of fruits, vegetables, meats, spices, processed foods, plastics, inks, medical wastes and paper; and for the sterilization of insects (Farrar et al., 1993). The different dose measurement technologies include ceric-cerous sulfate, radiochromic films, polymethylmethacrylate (PMMA), radiochromic optical waveguide, dichromate, calorimetric, radiochromic liquid solution, ethanol-chlorobenzene, and cellulose triacetate dosimetry systems (Farrar et al., 1993). Because of their specificity, only the dosimeters used in this study are reviewed in this document.

Table 2.2

Food products approved for irradiation in the United States

Food Product	Agency and approval date	Purpose for Irradiation	Maximum permitted dosage (kGy)
Wheat and wheat powder	FDA – August 21, 1963	Insect disinfestations	0.20 to 0.50
White potatoes	FDA – July 8, 1964	Inhibit sprout development	0.05 to 0.15
Spices and dry vegetables	FDA – July 5, 1983	Microbial disinfection and insect disinfestations	10.0
Dry or dehydrated enzyme preparations	FDA – June 10, 1985	Microbial disinfection	10.0
Pork carcasses or fresh nonheated processed cuts	FDA – July 22, 1985	Control *Trichinella spirallis*	0.30 to 1.0
Fresh foods	FDA – April 18, 1986	Delay maturation	1.0
Dry or dehydrated aromatic vegetable substances	FDA – April 18, 1986	Microbial disinfection	30.0
Fresh, frozen uncooked poultry	FDA – May 2, 1990 USDA – October 21, 1992	Control food-borne pathogens	3.0
Refrigerated and frozen uncooked beef, lamb, goat and pork	FDA – December 3, 1997 USDA – February 22, 2000	Control food-borne pathogens and extend shelf-life	4.5 (refrigerated) 7.0 (frozen)
Fresh shell eggs	FDA – July 21, 2000	Control *Salmonella*	3.0

Adapted from GAO (2000).

1.0 kGy = 1,000 Gy.

With electron beam systems the shape of the product may alter the absorption of the radiation, which is mono-energetic and might introduce variability in the absorbed dose; therefore, care must be taken to deliver the dose as uniform as possible, for which experimental samples are sometimes placed in rotating tables, and the dosimeter must be appropriate for both the dose range and the operating temperatures (Thayer, 2000). To enable replication of experiments it is necessary to report the variability of the absorbed dose along with type of incident radiation, dose rate, and environmental factors to which the sample is exposed during and after irradiation, such as temperature and atmosphere (Thayer, 2000). For example, Thayer (2000) estimated an error of 1.7% in measured dose using reference dosimeters supplied by the National Institute of Standards and Technology (NIST) at doses of 10, 25, and 40 kGy, when in practice it could be at least 3%.

The concept of exposure to radiation, which applies to electromagnetic radiation, is defined in terms of the amount of ionization they produce (Turner, 1995). The unit of exposure is called the roentgen (R), defined as the amount of radiation that produces in air 1 esu of charge of either sign per 0.001293g of air (mass of air that occupies 1 cm^3 at standard temperature or pressure). This concept provides a practical measurable standard for electromagnetic radiation and can be computed from the average energy needed to make an ion pair or W (34 eV/ip = 34 J/C); therefore, an exposure of 1 R in air gives a dose in air of 8.8×10^{-3} Gy or 0.88 rad (1 R = 2.58×10^{-4} C/kg x 34 J/C = 8.8×10^{-3} J/kg). It has been shown that a radiation of 1 R would produce a dose of 9.5×10^{-3} Gy (= 0.95 rad) in soft tissue (Turner, 1995). Thus, in radiobiological experiments, the measurement

of the dose or dose rate in a beam can be accomplished by measuring the current from an ionization chamber placed in the depth position where the target sample will be exposed to the beam (Turner, 1995).

Radiochromic films were developed for the measurement of absorbed dose and the mapping of radiation fields; they develop a characteristic color upon exposure to ionizing radiation and become progressively darker in proportion to radiation dose. This change can be measured with any densitometer, scanner or spectrophotometer. The color change is the result of polymer production in the active layer of the film and usually is cyan blue (ISP, 2004).

When dose measurement is complicated by the complex geometry of the target, other applications such as the Monte Carlo simulation can be applied successfully for the determination of surface dose distribution on fruits (Brescia et al., 2003).

2.3.5 Biological effects of radiation

The biological effects of radiation (such as DNA damage, injury, and repair) can be quantitatively described in terms of dose-response relationships, that is, the incidence or severity of a given effect, expressed as a function of dose (Turner, 1995).

The response of living cells, as bacteria, depends on the type of radiation applied. Radiation of high Linear Energy Transfer (LET), or ionizing density, is more effective than radiation of low LET ($<10keV/\mu m$). This effectiveness depends on the radiation quality, dose rate, and dose fractionation. Cells can repair the damage caused by radiation at low dose rates that would be lethal if received in a shorter period of time (Turner, 1995). In other words, higher dose rate should be more lethal than low dose

rate, because there is no chance to repair.

Loss of biological function in bacteria by the destruction of nucleic acid targets occurs by single hit action at any rate, and since cellular DNA is attached to cell membranes (where the initiation of DNA synthesis occurs), their integrity is critical for the bacteria viability (Alper, 1977). For the killing effects of ionizing radiation on bacteria, it is assumed that DNA is the main target molecule, and that primary lesions are single and double strand breaks, which are caused by direct actions induced by primary events in these molecules as well as indirect actions of intermediates such as free radicals induced by primary events in the medium. Therefore, cell killing is due to: production of effective primary events in the target, induction of primary lesions in the target molecule, and induction of decisive lesions in the target molecule (Iwanami and Oda, 1985). The effects of neutrons and X-rays in damaging lysosomal membranes where found to be less effective at higher LET because the intracellular targets of radiation include macromolecules or structures like enzymes, nucleic acids or membranes which evidence supports that are inactivated by single hit action (Alper, 1977).

An inverse correlation was observed between the guanine-cytosine (G + C) content in a series of bacterial species and their sensitivity to X-ray inactivation as ultraviolet light, but the repair mechanism may vary among species (Ginoza, 1967). The species studied can be arranged by their G + C content as listed in the *Bergey's Manual of Systematic Microbiology* (Kreig, 1984) as: *Enterobacter aerogenes* (53 to 54%), *Salmonellae* (50 to 53%), *Escherichiae* (48 to 52%), *Listeria monocytogenes* (37 to

39%), and *Listeria innocua* (36 to 38%). For reference, the G + C content of *Micrococcus radiodurans*, the most resistant bacteria to radiation so far, is 70-75.5% (Kreig, 1984).

2.3.5.1 Reported radiation D_{10} *values*

A comparison between gamma and electron beam treatments (in the range from 0 to 2 kGy) was made by Miyahara and Miyahara (2002) using anaerobic colony suspensions of Bacillus cereus, *E. coli* 0157, *Listeria monocytogenes*, *Salmonella Enteritidis*, and *Clostridium Perfringes* suspended in saline solution (10^{-7} concentration) under anaerobic conditions. The authors concluded that electron beam irradiation reduced *Bacillus cereus* and *Escherichia coli* populations more effectively than gamma but without statistical difference and no differences for *Listeria*, *Salmonella*, and *Clostridium* between irradiation sources. Differences between sources were noticed when *Bacillus* cereus was incubated at 4ºC before irradiation. They obtained D_{10} values of 0.46 kGy (gamma irradiation), 0.36 kGy (electron beam) and 0.53 kGy (stored at 4ºC before treatment, using gamma irradiation). Also, D_{10} values (kGy) ranging from 0.24 to 0.31 kGy for *E. coli* O157:H7, 0.507 to 0.61 kGy for *L. monocytogenes*, and 0.62 to 0.80 kGy for *Salmonella* in beef, were reported by Thayer et al. (1993), considering different combinations of fat level and treatment temperature (-17ºC to 15ºC) using gamma irradiation. Thayer and Boyd (1993) reported D_{10} values of 0.27 kGy at 5ºC and 0.42 kGy at -5ºC in chicken meat, when gamma irradiation was applied. Huhtanen et al. (1989) obtained D_{10} values of 0.27 kGy (± 0.04) at doses between 0 and 0.5 kGy and 0.35 kGy (± 0.09) at doses of 1.0 to 2.0 kGy from seven strains of *Listeria* subjected to

gamma irradiation in a mixture of 0.4 % Nutrient Broth (NB) and 1.5 % Tryptic Soy Broth (TSB). D_{10} values in food systems with nutritional conditions similar to fruits were not found. Using four *L. monocytogenes* and two *L. innocua* Kamat and Nair (1996) determined a D_{10} value (gamma radiation at 0.05 kGy/min in the range from 0 to 2.0 kGy) of 0.5 kGy in chicken meat homogenate. Selected results reported in ICMSF (1996) from different authors, for different strains in systems similar to the proposed, suggests that the highest expected D_{10} values for *E. coli*, *Listeria monocytogenes* and *Salmonella typhimurium* are 0.30, 0.50 and 0.59 kGy respectively. These results lead us to conclude that there are differences in D_{10} values not only among strains, but due to irradiation sources, food systems and environmental conditions (Appendix A).

2.3.6 Chemical effects of radiation

The changes induced in food by ionizing radiation can be the result of direct action, if a sensitive target such as the DNA of a living microorganism is damaged directly; or indirect action, if the changes on food are caused by the products of water radiolysis (Stewart, 2001). Because all living matter is composed of elements such as carbon, hydrogen, oxygen, and nitrogen, it is also important to understand that when ionizing radiation passes through this matter it loses energy that once absorbed results in chemical changes (Stewart, 2001). In the case of water, this results in reactive entities called free radicals; these reactions can be influenced by the presence or absence of oxygen resulting in the formation of different radicals like •OH, •H, and e⁻, and other secondary products as hydrogen peroxide (H_2O_2), and hydrogen (H_2).

2.3.6.1. Carbohydrates

When sugars are irradiated in the solid state, their melting point decreases, their optical rotation is reduced, and browning can be observed. For example, irradiation of fructose, galactose, glucose, and xilose results in the formation of a mixture of gases, particularly hydrogen (H_2) and carbon dioxide (CO_2) with traces of methane (CH_4), carbon oxide (CO), and water (H_2O); also, the radiolytic products of water will have a significant influence on the nature of the radiolytic products formed on radiation of sugars (Stewart, 2001).

It is known that irradiation leads to the degradation of polysaccharides such as starch, cellulose, and pectin, but the presence of other food constituents (like lipids, proteins or vitamins) might exert a protective effect on carbohydrates during radiation (i. e. the casein micelle which is a composite structure); thus, the effects observed when pure solutions of sugars are irradiated may not necessarily be noted when the sugars are contained within a foodstuff (Stewart, 2001).

2.3.6.2. Proteins

In the case of proteins, the nature of the radiolytic products is dependent on the amino-acid; in general, sulfur-containing amino-acids and aromatic amino-acids are the most sensitive to irradiation, being the cause of this sensitivity the presence of a thiol or disulfide group that leads to oxidation and degradation of the –SH and –S-S- groups in the first, and the hydroxylation of the aromatic ring in the second. Radiation-induced reactions in proteins are strongly influenced by their complex structure as the folding of

the peptide chains, disulfide links between chains, secondary binding forces, hydrophobic bonds, ionic bonds or those holding several subunits together as a functional protein (Stewart, 2001).

2.3.6.3. Lipids

Changes in lipids due to ionizing radiation can be brought by catalyzing their reaction with molecular oxygen, or by the action of high energy radiation on lipid molecules, reactions that are affected by parameters such as the composition of the lipid (saturated or unsaturated), the presence of other substances (antioxidants), whether the lipid is in liquid or solid form, the radiation conditions employed, and the storage conditions after treatment. Other factors to consider are that the radiolysis of natural fats is significantly more complex compared with model systems, due to the large number of different fatty acids and the variation in their distribution on the glycerol molecules; if oxygen is present during or after irradiation, normal auto-oxidation is accelerated (Stewart, 2001).

2.3.6.4 Micronutrients

Sensitivity of micronutrients to ionizing radiation differs. In the case of water soluble vitamins the order in sensitivity is: thiamine (B_1), ascorbic acid (C), pyridoxine (B_6), riboflavin (B_2), Folate and niacin, and cobalamine (B_{12}); and fat soluble vitamins: α-tocopherol (E), carotene, retinol (A), cholecalciferol (D), and menadionine (K), from most to least sensitive respectively (Stewart, 2001).

Kattel (1997) evaluated the effects of gamma irradiation on the chemistry of

systems prepared with natural and simple sources of food constituents (at doses up to 20 kGy). No changes in pure fatty acids and canola fatty acid profiles were observed. The effect on the minor constituents was more significant than on the major constituents of casein and non-fat dry milk protein solutions, and a significant reduction of pH with fructose, sugar, starch and rice with increasing irradiation dose was observed.

A fruit model was developed by Beyers et al. (1983) to evaluate the effects of irradiation on subtropical fruits (mangoes and papayas), based in the major chemical constituents of subtropical fruits (carbohydrates), which in order of importance are: fructose, glucose, maltose and sucrose. The authors cite several studies that shown that irradiated sugar solutions are cytotoxic to plant cells and microbial systems, and mutation in *Salmonella typhimurium* due to exposure to irradiated sugar solutions (1% sucrose, glucose and ribose) is possible.

Beyers et al. (1983) observed that when irradiated (using gamma source) the five sugars present in mango (fructose, glucose, sucrose, maltose and ribose) were mutagenic towards *Salmonella Typhimurium* TA100 in the presence and absence of oxygen (with exception of fructose in anaerobic conditions), while there was no mutagenic response towards *Salmonella Typhimurium* TA1535, TA1537, TA1538, and TA398. After these results a study to evaluate synthetized radiolytic products of the sugars on *Salmonella Typhimurium* was conducted by the cited authors. Glyoxal and d-arabiono-hexos-2-ulose (glucosone) were mutagenic at high concentrations towards strain TA100. It was concluded that glucosone is mutagenic only under these specific experimental conditions, for a specific strain, and at least 20 to 200 times more or less toxic than the

values expected at 1.0 kGy. These results suggest that irradiation of food model systems that contain sucrose concentrations might have a mutagenic effect on certain bacteria strains.

Schubert and Sanders (1971) and Schubert (1973) evaluated the effects of chemical compounds (sucrose, glucose, fructose, mannose, L-rhamnose, galactose and fucose) on the inhibition of *Salmonella Typhimurium* LT2. In oxygen free solutions, the inhibitory action increased with dose (gamma irradiation) and it was enhanced two or three times when the solutions were autoclaved (121ºC, 20 min at 15lb/inch^2 or 10.35 Pa). The same studies found that other compounds such as dyceraldehyde and 2-deoxy-D-ribose developed antibacterial activity upon heating. Namiki et al. (1973) evaluated the antimicrobial effects of irradiated glucose, fructose and sucrose solutions in doses up to 10 kGy, and demonstrated that these solutions showed antibacterial activity towards *E. coli*. The antibacterial activity was reduced by autoclaving (121°C, 20 min at 10.35 Pa), leading to the conclusion that the activity was not due to peroxides formed by radiolysis but rather to a more thermo-stable product. Other experiments with *Salmonella Typhimurium* strain TA100 in different sugars solutions (glucose, fructose, sucrose, maltose and ribose) under gamma irradiation (dose up to 50 kGy) did not result in mutagenic responses (Beyers et al., 1983).

2.4 Predictive microbiology

The basis of predictive microbiology is that a detailed knowledge of microbial responses to environmental conditions enables an accurate evaluation of the effect of processing, distribution and storage operations on the microbiological safety and quality

of foods (McKeein et al., 2001). Kinetic parameters and models are used for the development of food preservation processes to ensure safety and permit the comparison of different process technologies on reduction of microbial populations (FDA/CFSAN, 2000). One specific application is the development of microbial growth and survival models.

When used in the field of microbiology, "stress" refers to any factor or condition that adversely affects microbial growth or survival; "mild" describes stress levels that do not result in viability loss but reduce growth rate; "moderate" decreases microbial growth and causes some loss of cell viability; and "extreme" or "severe" is a level that is normally lethal and results in the death of the majority of the population (Yousef and Courtney, 2003). Based on these terms, microorganisms subjected to "stress" respond in various ways: changes in the membrane fluidity, alteration of cell protein structure or disruption of ribosomes, or affects nucleic acids, which is the main effect of radiation. This response may result in the production of protein that repairs, maintains or eliminates damage, increases the resistance or tolerance to deleterious factors, transformations to a dormant state (spore formation or passage to a viable but not culturable state), evades host organism defenses and adaptive mutations. Therefore, this understanding will lead to a more accurate characterization of the different populations produced after "stress" is applied to microorganisms.

Legan and Vandeven (2000) defined growth models as those concerned with responses where at least part of a range of conditions permits growth to occur. The microbial response can be then described as the increase in numbers with time (kinetic),

the conditions allowing growth (boundary) or the chance of growth (probabilistic). The authors state that for experimental design of kinetic growth models is necessary to understand the following concepts: (a) factor, which is an independent variable or design condition as temperature or pH that takes more than one value; (b) treatment, a unique combination of factors and their levels like pH 6.5 and 25ºC; (c) response, known as the dependent variable and is what is measured, e. g. viable count; and (d) parameter, a term in a model that is applied to the value of a factor to obtain the prediction.

2.4.1 Kinetics of microbial growth

2.4.1.1 Survival

The traditional approach to describe the survival of microorganisms as function of time can be expressed in terms of a survival curve (FDA/CFSAN, 2000),

$$\log \frac{N}{N_o} = \frac{-t}{D} \tag{2.1}$$

The corresponding model from chemical reaction kinetics is described by the first order kinetic model,

$$\frac{dN}{dt} = -kN_o \tag{2.2}$$

where k is a first order reaction rate constant or the slope of the natural logarithm of survivors with respect to time. Eq. (2.2) can be integrated to obtain the expression for logarithmic reduction of microbial populations,

$$\ln \frac{N}{N_o} = -kt \tag{2.3}$$

A comparison of Eq. (2.1) and Eq. (2.3) yields the relationship between the decimal reduction time (D) and the first order reaction rate constant (k),

$$k = \frac{2.303}{D} \tag{2.4}$$

The D values are used to describe the logarithmic reduction in a specific microbial population at a constant and defined temperature, pressure and/or electric field (FDA/CFSAN, 2000). Therefore, a D value should be stated providing an estimate of uncertainty as a description of the biological and physical conditions under which the value was obtained (Thayer, 2000). It is the slope of the line between *A* and *O, or A* and *Su*, when a treatment is applied at the same rate (Figure 2.1). Ideal death curves involve 10-12 points over a 6 to 7 $\log_{10}$ (or greater) reduction in population size, which implies an inoculation level of at least 10^8-10^9 CFU/ml. A zero time or dose is essential, and their intervals increasing geometrically can be beneficial (Legan and Vandeven, 2000).

The D values for thermal inactivation are expressed as the number of minutes at a given temperature that are required to inactivate 90% of the cells of an organism; while D_{10} values for inactivation of bacteria by irradiation are expressed in terms of the dose of ionizing radiation that is actually absorbed, which is expressed in terms of the gray (Gy) or the absorption of 1 joule of energy by each kilogram of matter through which the energy passes (Thayer, 2000).

Microbial decimal reduction due to irradiation (D_{10} values) can be defined as the

dose in kGy required for one logarithmic reduction of the initial microorganism population (ICMSF, 1980). This parameter is calculated from the negative inverse of the slope from the linear regression between the dose (kGy) and the logarithm of the microbial population (*A* to *B*, Figure 2.1).

The D_{10} method is the result of a target theory developed to give a mechanistic interpretation to the shape of microorganism survival curves. The theory explains the damage to the target cell (Brescia, 2002). Turner (1995) cite that cell inactivation is conveniently represented by plotting the natural logarithm of the surviving fraction of irradiated cells as a function of the dose they receive, and a linear semilogarithmic survival curve implies exponential survival of the form,

$$\frac{S}{S_0} = e^{-D/D_0} \tag{2.5}$$

Eq. (2.5) can also be expressed as (Brescia, 2002),

$$\frac{N}{N_0} = e^{-kD} \tag{2.6}$$

Eq. (2.6) is also a direct result from Eq. (2.2), meaning that an increment of dose (*dD*) will result in a decrease of *N* by *dN*. If the fraction of surviving cells is *S*, then,

$$S = e^{-kD} \tag{2.7}$$

In the single target single hit survival model, an additional dose produces an exponential decrease with slope *-1/D₀*, or the negative reciprocal of the slope (Figure 2.1).
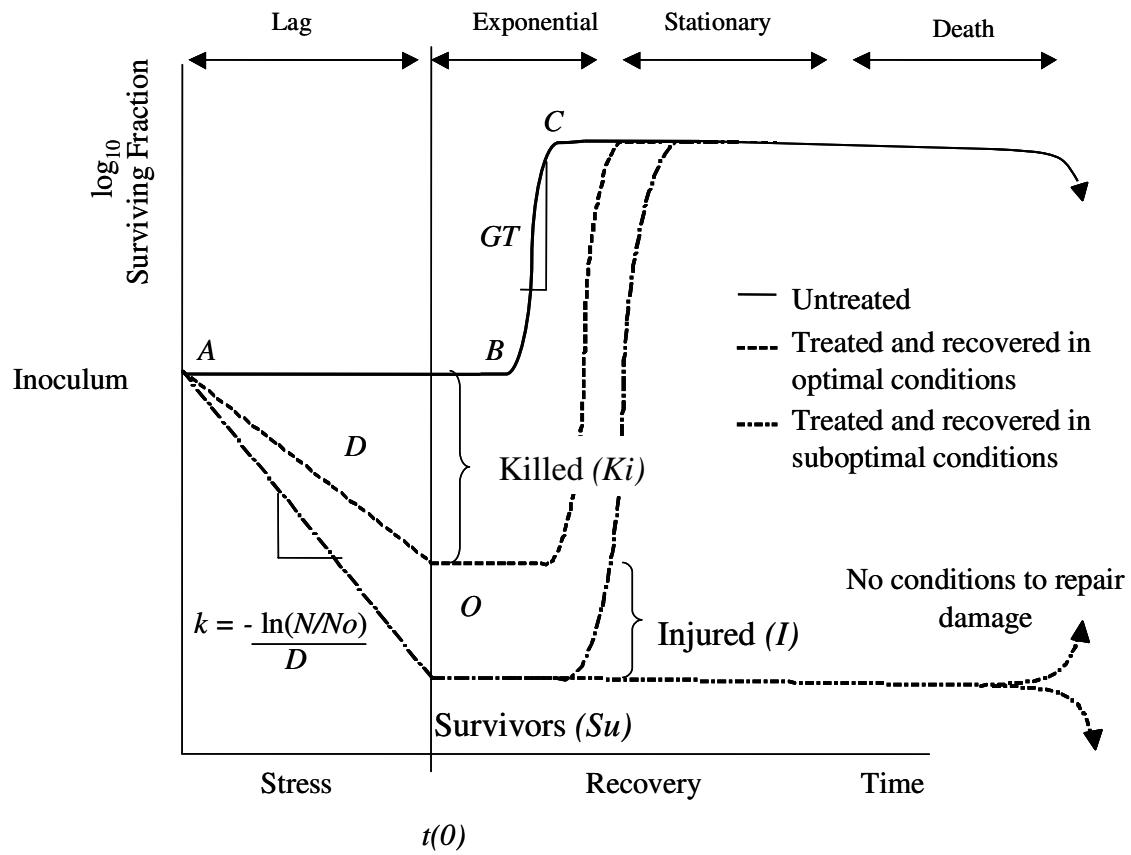
Figure 2.1. Theoretical destruction-repair curve. (Adapted from Mossel and Netten, 1984).

Assuming that the distribution of hits follows the poison distribution[1], the proportionality constant k is equal to $-1/D_0$, where D_0 is the dose that results in an average of one hit per target. If $D/D_0 = 1$, then $e^{-1} = 0.37$, and D_0 can be called the one hit per target, 37% of the original number of 37%, the e^{-1} dose, or "D-37" dose. (Brescia, 2002).

A model that yields a survival curve with a different shape is the multi-target, single-hit model, where the probability that all n targets in a cell are hit is $(1 - e^{-D/D0})^n$, in which case the cell is inactivated (Turner, 1995).

Therefore, the survival probability is,

$$\frac{S}{S_0} = 1 - (1 - e^{-D/D_0})^n \qquad (2.8)$$

when $n = 1$, Eq. (2.8) is reduced to Eq. (2.5), or the single-target, single-hit.

The multi-hit theory postulates that some systems contain a single target, which must be hit m times in order to inactivate the system, and units receiving $m - 1$ or fewer hits will survive (Brescia, 2002).

The survival probability in the multi-hit theory is described by Eq. (2.9) and Eq. (2.10).

---

[1] Probability distribution that describes all random processes whose probability of occurrence is small and constant. $f(x) = \dfrac{e^{-\lambda}\lambda^x}{x!}$ such that λ is the mean success in a given time, and x is the number of successes we are interested in.

$\dfrac{S}{S_0}$

1

0.1

0.01

Dose
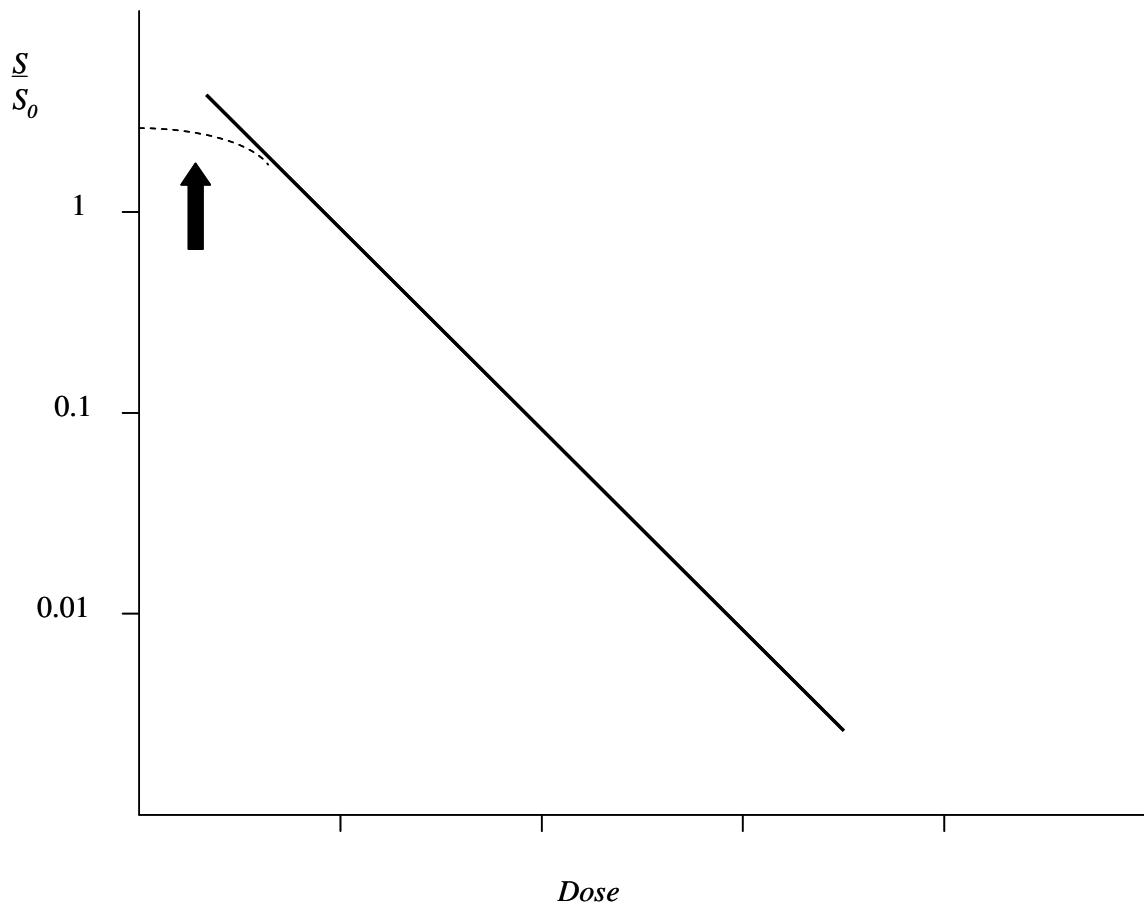
Figure 2.2 Theoretical survival curve. (Adapted from Turner, 1995).

$$S = e^{-kD}\left(1 + \frac{kD}{1!} + \frac{(kD)^2}{2!} + ... + \frac{(kD)^{(m-1)}}{(m-1)!}\right) \tag{2.9}$$

$$S = e^{-kD}\sum_{i=0}^{m-1}\frac{(kD)^i}{i!} \tag{2.10}$$

Other models have been investigated, as modifications to the multi-target, single-hit model that postulate that only any $m < n$ of the cellular targets need to be hit in order to produce inactivation (Turner, 1995).

Many studies estimate the D_{10} value and its variability from the slope of the linear portion of the inactivation curve, and shoulder effects are eliminated by not including the zero dose in the least squares analysis of the regression. Doses must be selected to provide at least five points in the regression, which variability estimate is influenced more by the number of doses than by replication; also, D_{10} values must be compared by analysis of covariance rather than by comparing means (Thayer, 2000).

Generally, all microorganisms are killed as an exponential function of absorbed dose although in some cellular systems there is a pre-exponential region, where at low doses the cells are more resistant to radiation. This has been attributed to repair or to the accumulation of sub-lethal damage (Adams and Stratford, 1977). The black arrow pointing the curved region in Figure 2.2 represents this effect. Among the main factors that affect this pre-exponential stage or alter the exponential response of organisms to radiation is the rate at which the cells are irradiated (Adams and Stratford, 1977).

2.4.1.2 Growth

The time that a single cell takes to divide into two is called the *Generation Time* (*GT*). In practice this term is referred to as the doubling time for the entire population (Ray, 2004). In a population of microbes, not all cells divide at the same time or at the same rate; therefore, this parameter provides valuable information for developing methods to preserve foods under different conditions, where in general it is assumed that under optimum conditions of growth, bacteria have the shortest *GT* (Ray, 2004).

When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression (Todar, 2002). For example, if we start with one cell, when it divides, there are 2 cells in the first generation, 4 cells in the second generation, 8 cells in the third generation, and so on. *GT* can be calculated from any portion of the growth curve; but the general rule is to determine it from the exponential part (from *B* to *C*, in Figure 2.1) of the curve.

Therefore, an expression of growth by binary fission is given by,

$$C = B \times 2^n \tag{2.11}$$

By definition, *GT* can be expressed as,

$$GT = \frac{t}{n} \tag{2.12}$$

Solving for *n*, the number of generations (organisms born from a mother organism) will be given by Eq. (2.13).

$$n = \log C - \log B \tag{2.13}$$

$$n = \frac{\log C - \log B}{\log 2} \tag{2.14}$$

$$n = \frac{\log C - \log B}{0.301} \tag{2.15}$$

$$n = \frac{3.3 \log C}{B} \tag{2.16}$$

Solving for *GT*,

$$GT = \frac{t(3.3 \log C)}{B} \tag{2.17}$$

For example, using Eq. (2.17) we can calculate the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth as,

$$GT = \frac{t(3.3 \log C)}{B}$$

$$GT = \frac{(4h)(3.3 \log 10^7)}{10^4}$$

$$GT = \frac{(4h)}{3.3 \times 3}$$

$$GT = 0.4h$$

Additionally, *GT*, also known as doubling time (t_d), in hours, can be determined from the named Gompertz equation (Ray, 2004),

$$GT = t_d = \frac{0.3t}{B - C} \tag{2.18}$$

As pointed by McKellar and Lu (2004) the Gompertz equation has a mechanistic interpretability and is enough to describe microbial growth when detailed information is not available. Other models (for example, the Baranyi model, Hills model or the Buchanan model) can accurately represent the results but more data is needed (McKellar and Lu, 2004).

Growth curves typically have 10 or more data points and the placement of points can be more important than the number in order to identify regions of rapid change (Legan and Vandeven, 2000). For accurate estimates of the length of the lag phase (*L* or *A* to *B*) and the rate of growth in the logarithmic phase (*B* to *C*) it is important to have data points close to the point of inflection that marks the transition between the two phases (Figure 2.1).

Where the constant *e* is the natural logarithm of 2.71828, or ln (2.71828) = e^1. The lag phase (*L*) can be described as,

$$L = t_B - t_A \tag{2.19}$$

where t_B is the time when a microbial population begins the exponential phase and t_A the inoculation time (point *A*) or after a treatment (*O* or *Su* or *t(0)*) as in Figure 2.1.

For kinetic growth studies, inoculum's size, growth rates, generation times, maximum populations, and conditions (i. e. temperature, water activity, pH) are important. Inoculation to give an initial concentration of 10^2-10^3 CFU/ml is ideal because it allows counts to be measured during the lag phase but reduces the risk of unrealistic raising the probability of growth as it can happen with high inoculation levels. It also approximates the concentration of pathogens expected in foods (Legan and Vandeven, 2000).

The growth factors (water, energy, carbon, nitrogen and minerals source) affecting the multiplication of microorganisms can be described mathematically. For instance, Choisy et al. (2000) described the growth rate of microorganisms for low concentrations of one food constituent (limiting food) under specific conditions such as pH and temperature using the following equation,

$$T = T_{\max} \frac{[S]}{K + [S]} \tag{2.20}$$

2.4.1.3 Injury

Injury may be manifested by an inability of microorganisms to form colonies on a defined minimal medium while retaining the colony forming capabilities when complex nutrients are present in the medium (Busta, 1978). Therefore, the loss in tolerance to the restricted conditions of the selective agent in the test platting agar would be interpreted as sub-lethal injury of the stressed cells since no inactivation is observed when the samples are enumerated on the base platting agar.

Cells are classified as injured rather than dead when they have the capability to function in an unrestrictive environment and can regain a normal physiological state concomitant with initiation of growth and cell division. The factors that influence the growth of damaged cells include: specific nutrients, pH, temperature, gaseous atmosphere, culture age, redox potential, osmolality, water activity, ionic strength, salts, surface tension, and storage (Busta, 1978). In the case of radiation, the greater the dose, the greater the percentage of injury that will occur (Thayer, 2000).

Studies on the recovery of two species of *Escherichia coli*, four species of *Listeria*, and one specie of *Salmonella* after gamma irradiation treatments (0 to 3.0 kGy at 1.22 kGy/h) established a recovery protocol, where Basal Yeast Extract Agar (BYEA) is the optimum and Tryptic Soy Agar (TSA) the restricted growth medium (Lucht, et al., 1998). The authors also introduced the concept of Recovery Factor (*RF*), which is a dimensionless factor, calculated as the ratio of populations recovered in optimum (*R*) media (CFU/ml) to those recovered in restricted (*r*) media (CFU/ml),

$$RF = \frac{R}{r} \tag{2.21}$$

An effective treatment protocol can be determined from the study of microbial dose-response under detrimental and optimal growth conditions. This study implies the analysis of variables related to energy deposition (dose, dose rate, type of radiation) and chemistry of the product (temperature, physical state, nutritional content), which allow the determination of the range for optimization or detriment of conditions for microbial growth (Borsa, 2004).

A typical Destruction-Repair Curve (Figure 2.1) shows the four growth phases (lag, logarithmic, stationary and death) for untreated microbial populations, treated at the same dose rate and recovered under suboptimal conditions, and those treated at the same dose rate and recovered under optimal conditions.

From this graph the following parameters can be determined:

(1) D values at the same dose rate, calculated from the slope of the linear region (A to $Su,$ or A and O depending on the media),

(2)   The Lag phase (L) or time required for bacteria to adapt to the new environment (B - M),

(3)   The number of healthy survivors (Su) in $\log_{10}$ CFU per milliliter, gram, or unit area (depending on the sample and the method that is used) can be determined by measuring the growth of bacteria under suboptimal conditions,

(4)   The number of injured (In) bacteria, calculated from the growth between optimal conditions and suboptimal conditions after treatment (O - Su),

(5)   The number of those bacteria killed (Ki), calculated from the difference between the inoculum size, A, (or untreated population) and the population recovered under optimal conditions, O (A - O).

2.4.2 Measurement of microbial populations

Colony count methods provide an estimate of the number of viable microorganisms in a food sample according to the medium employed and the time and

temperature of incubation. It is necessary to shake and dilute samples to uniformly distribute bacteria before inoculation in agar plates, where each colony that appears on can arise from a clump or a single cell, and should be referred as Colony Forming Unit (CFU). In colony count methods, precision and accuracy are important, being the first the ability to obtain similar results when repetitive counts are made by the same or different person and the second the difference between the counts obtained and a true count (Busta et al., 1984). Therefore statistical analysis plays an important role in microbiological tests.

Dalgaard et al. (1994) reported that although many studies determine the slope from the logarithm of the relationship between optical density and time during the apparent part of the growth rate, this method is consistent with modified Gompertz-transmittance estimates (that describes the growth of bacteria populations based on spectroscopy), but requires an accurate calibration factor to relate with viable counts. These factors are not reported in the literature. Therefore, the best method to accurately measure microbial populations (in CFU/ml) is the Standard Plate Count (SPC) technique.

2.5 Microbial indicators and surrogates

The use of coliforms, or "generic" *E. coli*, as indicators of enteric contamination in other systems, such as potable water, will sometimes stimulate consideration for similar use in fresh produce. Indicators are marker organisms whose presence in given numbers points to inadequate processing for safety, and surrogate are nonvirulent strains of the target pathogen that retained all other characteristics except pathogenicity. These

microorganisms are invaluable in validating the efficacy of produce decontamination processes; their use derives from the need to prevent the introduction of harmful organisms in production facilities and laboratories. Therefore, the use of indicators and surrogates by processing companies is of great importance to ensure microbiological safety of the process (FDA/CFSAN, 2001b).

Among the considerations for a standard surrogate procedure for determining the efficacy of a process for control of pathogens on fruits and vegetables, as in any process, are: (1) selection of surrogate, (2) type of produce, (3) procedure for evaluating processing test condition, (4) retrieval of surrogates, and (5) reporting results. When selecting surrogates, the following characteristics are desirable: (a) non-pathogenic, (b) inactivation characteristics and kinetics that can be used to predict those of the target organism, (c) behavior similar to target microorganism when exposed to conditions similar to the processing parameters (pH, temperature, oxygen), (d) easy preparation, (e) genetically stable and susceptibility to injury similar to the target pathogen (FDA/CFSAN, 2001b). Other characteristics include: easy enumeration using rapids, sensitive and inexpensive detection systems, and easy differentiation from other micro flora. Usually, the strain is selected from the background flora by using a selective agent in the recovery media (Slade, 2003). To easily differentiate a good surrogate from the microflora, it should have a stable marker, for which antibiotic resistance can be used as selective criteria (Peri, 2003).

Some authors such as Thayer (2000) state that the first source of variability when determining D_{10} values is the choice of the isolate or isolates that will be used, for which

some researchers prefer to use a cocktail of 3 to 5 isolates hoping to find at least one that had similar resistance to that of the most resistant strain. The important factors that affect D_{10} values are: phase of growth (bacteria harvested in the stationary phase generally show more resistant), the past history of the strain (environmental factors as pH, temperature and oxygen alter resistance), and suspending medium (D_{10} values obtained in broths are generally lower than those obtained in a food product) (Thayer, 2000).

2.5.1 Previous studies using surrogates

Stress responses and cross protection of non-pathogenic *E. coli*, have been studied extensively suggesting that acid "habituation", nutrient starvation, and growth in the stationary phase yielded populations that were more resistant to the various stresses than control populations (Figure 2.1). The variation in resistance properties of non-pathogenic and pathogenic strains reinforce the importance of using resistant strains to evaluate the efficacy of food preservation treatments and developing process criteria, for which the measurement of *in vivo* expression of stress-related genes in food systems could be evaluated to be used as selection criteria (Johnson, 2003).

2.5.1.1 Escherichia coli

A study by Salter et al (1998) used a non-pathogenic strain (*E. coli* M23) as surrogate of a pathogenic strain of *E. coli* to develop a four-parameter model from the relation of temperature and growth rate. This non-pathogenic strain also proved to be a suitable surrogate under osmotic stress (Shadbolt et al., 1999). Pao and Davies (2001) compared the chemical resistance (immersion of inoculated oranges in a 500mL-bag of

pH 11-12) and thermal resistance (immersion of oranges in a hot water bath at 70 or 80°C) of six non-pathogenic strains as potential surrogates. The research demonstrated that two strains of *E. coli* from the American Tissue Culture Collection (ATCC 11229 and ATCC 25922) may be utilized as surrogates to conduct research in fresh fruits.

Among the strains cited in literature *E. coli* ATCC 25922 is one of the most currently used. Thayer and Boyd (1993) successfully determined that this strain (*E. coli* ATCC 25922) has the same sensitivity to gamma radiation than their pathogenic counterparts in meats (deboned chicken and ground beef). Leenanon and Drake (2001) compared two pathogenic *E. coli* O157:H7 (ATCC 43895), and an *rpoS* (a gene which was modified as in K-12) mutant (FRIK 816-3) with the non-pathogenic *E. coli* ATCC 25922 under heat resistance (thermal *D* values at 56ºC) before and after acid stress (culturing cells for 18 hours at pH 4.8 to 4.9), starvation (pelleting cells for 18 hours), and cold stress (freeze-thawing at -20ºC to 21ºC). The authors observed that the non-pathogenic strain showed the least heat resistance before and after acid stress, but starvation enhanced its resistance, while cold stress decreased the heat resistance in the three strains. Peggy et al. (2001) evaluated four potential surrogates for *E. coli* (ATCC 4351, ATCC 25922, FRIK 185, and FRIK 859) by comparing their thermo-tolerance (pH 3.3, 4.1 and 11, 14ºBrix apple cider heated under conditions ranging from 60ºC for 14 seconds to 71.1ºC for 14 seconds) and survival in apple cider with separate cocktails of *E. coli* O157:H7 strains (ATCC 43849, ATCC 43895, C7927, and USDA-FSIS-380-94), *Salmonella* (CDC 0778, CDC F2833, CDC H0662) and *L. monocytogenes* (H0222, F8027, and F8369). The authors concluded that the strain ATCC 25922 was the least

thermo-tolerant, and should not be used as a surrogate for *E. coli*, while FRIK859 was the most thermo-tolerant strain of all the *E. coli* species tested, and therefore a suitable surrogate under these treatments.

In a study to validate the sterilizing efficacy of ultraviolet (UV) pasteurization, Duffy et al. (2000) found that *E. coli* ATCC 25922 is a good surrogate for *E. coli* O157:H7. The conclusion was based on the fact that this non-pathogenic strain showed the same sensitivity to UV as the pathogen. This strain along with other *E. coli* strains (ATCC 23716 and ATCC 11775) were used by Sapers et al. (2000) to evaluate the efficacy of using hydrogen peroxide in apple washing treatments and were found to be suitable surrogates. The results of this study suggested that a particular non-pathogenic strain behaves differently under specific process conditions; then the selection of potential surrogates cannot be based on studies that use a different process technology. However, as suggested by some investigators, a standard strain should be used to compare different process technologies, which will help to evaluate the further elimination of bacteria with defined resistance.

Other strain used as an indicator and a possible surrogate is *E. coli* ATCC 11229. Blaser et al. (1986) compared the inactivation of *Campylobacter jejuni* by using chlorine and monochloramine water treatments. Because of the similarity of the results between the *E. coli* and *Campylobacter* strains the authors concluded that ATCC 11229 could be used as an indicator of inactivation of fecal contamination of *C. jejuni* in water. This non-pathogenic strain was also evaluated by Hoyer (1998) and Sastry et al. (2000) under UV treatments (254nm) of drinking water.

A well-studied non-pathogenic strain of *E. coli* is K-12 MG1655. When naming bacteria strains the initials included in the name represent the Institution that preserves them, or the person who isolated the strain, in this case Mark Guyer. *E. coli* K-12 MG1655 was sequenced because it approximates wild-type E. coli and "has been maintained as a laboratory strain with minimal genetic manipulation, having only been cured of the temperate bacteriophage lambda and F plasmid by means of ultraviolet light and acridine orange, respectively." (Blattner et al., 1997). The mutations of this strain, which are listed in the genotype are present in most K-12 strains and were probably acquired early in the history of the laboratory strain. A complete information about this strain is listed by EGP (2004), in brief they cite that a frameshift at the end of *rph* results in decreased *pyrE* expression and a mild pyrimidine starvation, such that the strain grows 10 to 15% more slowly in pyrimidine-free medium than in medium containing uracil. This citation states that the *ilvG-* mutation is a frameshift that knocks out acetohydroxy acid synthase II and the rfb-50 mutation is an IS5 insertion that results in the absence of O-antigen synthesis.

MG1655 was derived from strain W1485, which was derived in Joshua Lederberg's lab from a stab-culture descendant of the original K-12 isolate, which originally was obtained from a stool sample of a diphtheria patient in Palo Alto, CA in 1922 (EGP, 2004).

High pressure resistant mutants (LMM 1010, LMM 1020, LMM 1030) of *E. coli* K-12 were evaluated by Hauben et al. (1997) no significant differences in *D* and *z* values (*z* is the increase in temperature, in ºC, required to achieve one logarithmic reduction in

bacterial populations, in CFU/ml) at 58 and 60ºC, between LMM 1010 and their parent strain were found. However, the three mutants were less sensitive to pressure treatments (200 to 800 MPa for 15 minutes). These strains were evaluated by Masschalck et al. (2000) under pressure treatments (200 to 600 MPa during 15 minutes and interrupted treatments of 10 minutes three times) with the addition of lysozyme (50 µg/ml) and nisin (100 IU/ml) to the cell suspension. This study determined that the mutants were more sensitive to pressure treatments when the antimicrobial peptides were added. Applying interrupted treatments did not demonstrate a higher sensitivity of the mutants to pressure treatments. From these two studies, it can be said that mutants are not necessarily more resistant than their original culture; therefore, both organisms should be further evaluated. Peri et al. (2002) concluded that *E. coli* K-12 LM 1010 is a suitable surrogate for *E. coli* O157:H7 after evaluating their survival at extreme pH values (3.0, 3.5, 4.0, 9.5, and 10.0) and reduced water activity (below 0.92) (See appendix B).

From the evaluation of their survival in the skin of peaches, plums, and nectarines (using 4.5% horse serum as a dehydration protectant), Suslow et al. (2000) determined that *E. coli* 506 has the potential to be a suitable surrogate. Another potential surrogate is the non-pathogenic strain *E. coli* ATCC 35695, which was successfully used to evaluate washing treatments of apples by Annous et al. (2001).

The non-pathogenic strain *E. coli* CECT 516 did not show the same number of survivors when compared with two pathogenic strains of *E. coli* O157:H7 (CECT 4076 and 4267), under a range of storage temperature (22 to 4°C), and the presence of lactic acid bacteria in plain yogurt for 13 days. This result suggests that other microorganisms

might inhibit the growth of certain strains (Bachrouri et al., 2002).

Related studies using *E. coli* ATCC 25922 include the work by Hsu and Tsen (2001) who used the database GeneBank and the WSASP (1999) developed by Gene Computer Group Inc. (Madison, WI, USA) to design polymerase chain reaction (PCR) primers from malic acid dehydrogenase gene for detection in water and milk samples. This study can be used as reference for future work with such a strain, once the gene that gives resistance to irradiation is identified and altered. Because of the availability of the entire genome sequence, *E. coli* MG1655 was used by Janke et al. (2001) as a "driver" strain for hybridization and control in PCR reactions with the uropathogenic *E. coli* 536 to compare differences in pathogenicity. This study could be followed to determine the gene that gives the resistance of this strain to irradiation treatments.

2.5.1.2. Listeria

Listeria innocua, a non-pathogen, is the most frequent strain used as a substitute for the pathogenic *L. monocytogenes* in situations (such as food processing environments) where it would be undesirable to introduce pathogens (McKellar, 2003). A similar study (Kamat and Nair, 1996) used *L. innocua* F5646 and F5643 strains to compare the response with four *L. monocytogenes* strains (ATCC 35152, ATCC 35152 *½a*, L5458 *½b*, and L5562 *b2*) under heat, gamma radiation, lactic acid, and sodium nitrite treatments. The study determined that, for all the treatments, the survival responses of all the six strains were similar. Therefore, they can be used as biological indicators in meat (boneless chicken and red meat) processing treatments.

Studies using skim milk (Foegeding and Stanley, 1990; Fairchild and Foegeding,

1993) demonstrated that *L. innocua* M1 (ATCC 33091), a purified plasmid (pGK12) for antibiotic resistance, is a suitable biological indicator for the evaluation of pasteurization process lethality. This natural mutant strain is actually being used in raw skim milk to evaluate the effects of continuous flow processing on thermal inactivation. A more recent study in modeling high-temperature and short-time pasteurization of milk, compared *L. innocua* (LA-1) with *L. monocytogenes* (Pisayena and McKellar, 1999). The study found that the model developed with the surrogate was suitable for estimation of the survival of the pathogen in low water activity viscous products like concentrated milk (a_w 0.796-0.896). In a different study, Murphy et al. (2002) used the same modification to the strain (ATCC 33091) to evaluate thermal inactivation (55 to 70ºC) of five commercial meat products (chicken patties, tenders, franks, beef patties, and blended beef and turkey patties). The authors used *Salmonella* serotypes (*Senftenberg*, *Typhimurium*, *Heidelberg*, *Mission*, *Montevideo*, and *California*) as targets, and found differences in *D* and *z* (which is the temperature required to change the *D* value in 1 $\log_{10}$) values among species in all the five products. These results suggest that a surrogate cannot behave similarly in different products, and the study of similar processing technologies is a good reference for recent advances in the use of surrogates.

The growth of *L. innocua,* in several fruits (bananas, honeydew melon, cantaloupe, and passion fruit) was evaluated by Behrsing et al. (2003). The authors found that when inoculated at low levels (10^6 CFU/ml), this strain survived on the fruit skin when fruits were stored at different conditions (13 days at 18ºC, 1 day at 12ºC, 7 days at 8ºC; and 6 days at 10ºC respectively). *E. coli* (NCTC 10418) and *S. Salford*

(IMB 1710), two pathogens indicators of contamination of fruits, were used in the same study, were only the growth of *L. innocua* in cantaloupe was of particular concern. *L. innocua* was also used in cantaloupes, grapefruits and beets (Kozempel et al., 2002) to evaluate the application of vacuum/steam/vacuum in the surface of the fruits. Destruction levels ranged from 2.5- $\log_{10}$ reductions in CFU/ml in beets to 4- $\log_{10}$ reductions in grapefruits. The target, as in most of the disinfection technologies, was 5-$\log_{10}$ reduction.

In a similar study (Peri et al., 2002), it was determined that *L. innocua* 137 is a good surrogate of *L. monocytogenes* at low pH (3.0, 3.5, 4.0, 9.5, and 10.0) and reduced water activity (below 0.92).

2.5.1.3. Salmonella

Suslow et al. (2000) concluded that *Salmonella Typhimurium* LT2 was an appropriate bacterial surrogate to evaluate survival of similar gram-negative pathogenic bacteria (Enteric family) in fruits (peaches, plums, and nectarines). Other studies were conducted with this strain with similar results (Brocklehurst et al., 1995 a and b; Brocklehurst et al., 1997; Anderson et al., 2003)

Enterobacter species have been used as surrogate of *Salmonella* species by several researchers. Montville et al. (2001) used *E. aerogenes* B199A to evaluate gloves as a barrier for cross contamination between hands and food from chicken to lettuce while chopping. A similar study compared *E. aerogenes* B199A with *S. typhimurium* and *S. enteritidis* for cross-contamination by attachment to cutting boards and vegetables (cucumbers and lettuce) (Zhao et al., 1998). There were no significant differences (p >

0.05) between attachment and disinfection with alkyl dimethyl benzyl ammonium chloride-based kitchen disinfectant characteristics among the three species. Other specie that can be used as surrogate for *Salmonella* is *E. coli* K-12 (Peri et al., 2002).

Peri (2003) evaluated five potential *Salmonella* surrogates (*E. coli* K12 LMM 1010, *Enterococcus faecium* FAIR-E 151, *E. faecium* FAIR-E 225, *E. coli* K-12 ATCC 25253, and *L. innocua* 137) for resistance to pH (3.0 to 11.0), reduced water activity (0.92 to 0.84), and high temperatures (*D* values at 50 to 59˚C). The first two strains were found to be good surrogates under acid conditions and the last two strains were good surrogates in the alkaline range; while *E. faecium* FAIR-E151 and *L. innocua* 137 were good surrogates under reduced water activity.

In summary, no single strain could be used exclusively as a surrogate for all the conditions. Therefore, evaluation of potential surrogates under specific conditions is needed.

2.6 Effects of environmental conditions on microbial growth

Intrinsic factors of a food that affect microbial growth include water activity, pH and oxidatio-reduction potential, extrinsic factors are temperature, relative humidity and gaseous environment (Ray, 2004). There are specific gene products that control the expression of a group of genes that poise the cell for survival against a range of stresses such as heat, high osmolarity, low pH, peroxide stress, etc. depending on the bacteria. Jordan and Davies (2001) concluded that addition of sodium chloride (6g/100g) at pH of 5.5 and 6.0 had little influence in the growth rate of *E. coli* O157:H7, while at pH 5.0 the growth rate increased from 0.79 h^{-1} to 1.32 h^{-1}. The environmental limits for growth of

Escherichia, Listeriae, and *Salmonellae* species are listed in Table 2.3.

2.6.1 Fruit nutritional composition

Although fruit provides most of the essential nutrients, in general they are poor sources of protein or fat, and contain a very high percentage of their fresh weight as water (around 95% and as high as 98%) and starch or sugars depending on their maturity stage (Tucker, 1993). Ripening requires the synthesis of novel proteins and messenger ribonucleic acids (mRNA), as new pigments and flavor compounds which require energy and carbon skeleton building blocks that are supplied by respiration. The two major respiratory substrates found in fruits are sugars and organic acids, which differ in content within fruits (Tucker, 1993).

2.6.1.1 Fruit maturity stages

The main sugar transported from the plant leaves to the fruits is sucrose which is used for synthesis of pectic substances, cell wall materials, and the usual storage product: starch (Whiting, 1970). Most fruits accumulate the bulk of their carbohydrate content prior to the onset of ripening as starch, originated from photosynthesis and its assimilation differs during development and ripening; while some fruits as bananas store them as starch, others store them as sugars as tomato fruits (Tucker, 1993). The starch content is commonly found in the outermost cells of the fruit. Depending on the ripening process, fruits can be classified as: (1) those that accumulate their carbohydrates prior to ripening, can be harvested at the mature-green stage and still develop its sugar content, and have a peak in ethylene production after harvesting, which are called climacteric,

and (2) the ones that fail to develop sugars if harvested in the green stage which are called non-climateric (Tucker, 1993). In some climacteric fruits as bananas, mangoes and passion fruit, the increase in sugar production may continue up to maturity while in others an initial increase in sugars concentration is followed by a decrease (Whiting, 1970). The most common sugars in fruits are fructose, glucose and sucrose.

A summary of several studies conducted to determine the content of specific sugars in a variety of fruits (Sharaf et al., 1989; Agravante et al., 1990; Tucker, 1993; Wang, 1994; and Villanueva et al., 2004) is presented in Appendix C. The USDA Nutrient Database 2003 (USDA, 2004) reported the highest content of total sugars in tamarindo (66.0%) while olives had the lowest (0.54 %). The USDA study reported ranges from 25 to 95% water, 5.70 to 79.52% carbohydrates, 0.26 to 49.0% protein, and 0.04 to 15.41% lipids. Shallenberger and Birch (1975) and Holland et al. (1991) also reported that fruits may contain different levels and types of natural carbohydrates. The total content ranged from as low as 1.6% in lemon to as high as 65% in raisins. These studies suggest that if sucrose constitutes an average of 57.25% of the total sugars in fruits, tamarindo might contain up to 37.8% of sucrose; therefore a maximum of 40% of sucrose could be used to develop model fruits systems based on sugar content.

Among the climacteric fruits there is no pattern to describe the changes in sucrose, fructose and glucose concentrations between the growth and maturation stages. For instance, while some fruits such as apricots and peaches show an increase in sucrose content from green to maturity stages, other fruits such as passion fruit show a slight decrease, and even others like tomatoes show very little or none sucrose content when

Table 2.3

Growth limits for *Escherichia*, *Listeriae*, and *Salmonellae* species

Conditions		*Escherichia*	*Listeriae*	*Salmonellae*
Temperature (°C)	Minimum	7-8	-0.4	5.2*
	Optimum	35-40	37	35-43
	Maximum	44-46	45	46.2
pH	Minimum	4.4	4.39	3.8
	Optimum	6-7	7.0	7-7.5
	Maximum	9.0	9.4	9.5
Water Activity (a_w)	Minimum	0.95	0.92	0.94
	Optimum	0.995	NA	0.99
	Maximum	NA	NA	>0.99

NA: Not Available.

Adapted from ICMSF (1996).

*Most serotypes fail to growth at < 7°C.

ripe (Whiting, 1970). Even during storage there are differences in the changes of these three sugars in climacteric fruits. Mangoes and bananas hydrolyze completely their starch content during this period resulting in approximately equal amounts of glucose and fructose with little sucrose. Other fruits such as apricots and peaches show little changes during storage (Whiting, 1970).

Because of the variability within fruits in the pathways of starch degradation, and the resulting reducing sugars, even at the cultivar level, and their position inside the fruit, melons (*cucumis melo*) are a good choice for a model fruit since they do not have a significant amount of other carbohydrates (a potential source of energy for microbes) (Pratt, 1970). The fact that only the content of the three main sugars (sucrose, fructose and glucose) change during the ripening stages of melons makes it easier to develop a food model system that mimics the different stages of the fruit's maturity.

2.6.2 Sugar content in selected cucurbitas

Seymour and Glasson (1993) observed that sugar accumulation begins in melons during fruit development in both varieties (netted cantaloupe type and honeydew), with a rapid increase in the accumulation of sugars. The fruit reaches its full size, and it might comprise as much as 16% in the flesh of the fruit. Pratt (1970) stated that the usual limit (legal in California) for harvesting melons is no less than 10% sugars. The principal sugar accumulated within melons is sucrose, although high levels of fructose may be present in some cultivars. Sugar concentrations can vary in different parts of muskmelon and watermelon fruits (Pratt, 1970).

2.6.3 Food model systems

Food model systems are developed by scientists with different purposes. The dairy industry has been using food models to evaluate the use of ingredients and compare their functionality with natural products, especially on cheeses (Yang and Vickers, 2004). These models can be used as reference for the design of mixtures when different compounds are used (two or more).

The most common approach is to develop gel-based systems with specific ingredients added at different concentrations (Brocklehurst et al., 1995 a and b; and Brocklehurst et al., 1997). The growth of bacteria in gels with different sucrose contents (0% to 30% w/v) and changes in temperature (4 to 22ºC) has been evaluated using *Salmonella* LT2. Results suggest that the gels are a suitable media to study the behavior of microorganisms under different environmental conditions.

2.6.4 Limitations of using gel-based food model systems

2.6.4.1 Chemical reactions

Glucose is a monosaccharide (or simple sugar), a carbohydrate molecule that cannot be broken down to simpler carbohydrate molecules by hydrolysis. It can be joined together to form larger structures, namely, oligosaccharides and polysaccharides, that can then be converted into monosaccharides by hydrolysis. D-Glucose $(C_6H_{12}O_6 \cdot H_2O)$ is both a polyalcohol and an aldehyde, classified as an aldose (sugars containing an aldehyde group). When D-glucose is written in an open or vertical, straight-chain fashion, known as an acyclic structure with the aldehyde group at the top

and the primary hydroxyl group at the bottom, it is seen that all secondary hydroxyl groups are on carbon atoms having four different substituents attached to them. L-sugars are less numerous and less abundant in nature than are the D forms but nevertheless have important biochemical roles (Fennema, 1996).

In the other type of monosaccharide, the carbonyl function is a ketone group (ketoses). D-Fructose ($C_6H_{12}O_6$) is the prime example of this sugar group, and it is one of the two-monosaccharide units of the disaccharide sucrose. D-Fructose is the principal commercial ketose and the only one found free in natural foods; but like D-glucose, only in small amounts (Fennema, 1996).

All carbohydrate molecules have hydroxyl groups available for reaction. Simple monosaccharide and most other low-molecular-weight carbohydrate molecules also have carbonyl groups available for reaction. Because, in the process of oxidizing the aldehyde group of an aldose to the salt of a carboxylic acid group, the oxidizing agent is reduced, aldoses are called reducing sugars. Ketoses are also termed reducing sugars because, under the alkaline conditions of the Fehling test, ketoses are isomerized to aldoses (Fennema, 1996).

Common browning of foods on heating or on storage is usually due to a chemical reaction between reducing sugars, mainly D-glucose, and a free amino acid or a free amino group of an amino acid that is part of a protein chain. This reaction is called the Maillard reaction, and occurs when lysine is present; it constitutes 3.45% of the aminoacids of the gelatin composition (Bachman et al., 1974) and insignificantly increases upon irradiation (0.042%/1.0 kGy).

One of the major drawbacks encountered when preparing sugar solutions that require heating is the Maillard reaction. For instance, when milk is heated at temperatures above 100°C a series of reactions involving the aldehyde group of lactose and the α-amino group of protein-bound lysine occur (Singh and Creamer, 1992). This heat treatment results in changes in color, flavor, functionality and nutritive value. The overall reaction may be divided into three stages: (1) condensation of the reducing group of lactose (a reducing sugar) with the free epsilon amino group of lysine to form a Schiff's base, followed by the formation of an N-substituted glycosylamine; (2) a spontaneous and irreversible rearrangement of the glycosylamine via an Amadori rearrangement to form a 1-amino-1-deoxy-2-ketose; (3) reaction of the relatively stable Amadori compounds principally by two routes, this choice is affected by pH. At low pH, after enolyzation and further degradation, furfurals or hydroxymethylfurfurals are formed. At high pH, after enolization, elimination of the allyl amine residue and formation of a 1-methyl-2, 3-dicarbonyl intermediate, further descomposition forms a variety of compounds as pyruvaldehyde, diacetyl, hydroxydiacetyl, acetylfuran, pyrones and maltol.

Maillard reaction has a detrimental effect on the nutritive value of foods because the reaction of the sugar with the essential amino acid lysine becomes irreversible and biologically unavailable. Estimated losses in lysine in milk are 1-2% for pasteurization, 1-4% for UHT sterilization and 5% for brief boiling. The browning reaction can be inhibited by compounds as active sulphydryls, sodium bisulphate, sulphur dioxide or

formaldehyde, in practice, browning is controlled by limiting heat treatment, moisture content time and temperature of storage (Holsinger, 1997).

2.6.4.2 Microbes

In nature there is a myriad of species of microorganisms present in food materials that interact in symbiotic and antagonistic mechanisms. Therefore precautions must be taken when analyzing the results obtained from the disinfections of food model systems. For example, in cheese and yogurt lactic acid bacteria inhibit the presence of pathogens, other species may enhance their survival by forming benefic compounds (Cogan and Hill, 1993).

2.6.4.3 Irradiated food systems

An approach to predict the major chemical changes caused by irradiation in a real fruit is to prepare a system that contains all the chemical constituents present in a real fruit, subject it to irradiation and evaluate its wholesomeness (Beyers et al., 1983). This concept also applies to the growth of bacteria in these systems, where an accurate prediction of the presence of a nutrient and the effects of irradiation can be established. Because of the concern that toxic and/or clastogenic compounds are formed in irradiated foods, many researchers have used sugar solutions, instead of real fruits, to evaluate the biological activity and potential implications on the safety aspect of foods high in carbohydrate content (Beyers et al., 1983).

Schubert and Sanders (1971) reported the formation of radiolytic compounds (α, β – unsaturated carbonyls) after sugar solutions (0.058M) were subjected to gamma

irradiation, using a Cobalt-60 source at a wide range of doses (1.04 kGy to 20 kGy) at 4.02 kGy/h. The unsaturated carbonyls formation was enhanced by autoclaving (121ºC for 20 min and 10.36 Pa). These radiolytic compounds inhibited the growth of *Salmonella Typhimurium* LT2, but might have important implications to the safety of irradiated foods.

Namiki et al. (1973) evaluated the effects of irradiated pure sugars (1% of glucose, fructose and sucrose) on the growth of *E. coli* B, using gamma rays (from Cobalt-60), at 100 krad/h (1.0 kGy/h) as radiation source. A decrease from the initial microbial population, in viable cells (CFU/ml), was noticed in the fructose and glucose solutions (irradiated under aerobic conditions) during the first 24 hours while the inhibitory effect was not significant when the solutions where irradiated anaerobically (using nitrogen, N_2). Radiation D_{10} values were not reported, but these results suggest that there were no differences between 0 and 10 kGy for *E. coli* B grown in aerobically irradiated glucose and fructose solutions. There were not differences in microbial growth after irradiation when the pH of the three sugar solutions (under anaerobic conditions) was changed. However, a maximum growth was observed in alkaline conditions when the three sugars were irradiated under aerobic conditions at 1.0 Mrad (10 kGy). The authors concluded that heating, alkaline pH, and addition of catalase or ferrous ions inhibits the antimicrobial effect of sugars solutions when irradiated under aerobic conditions. The inhibitory effect of irradiated sugar solutions is not due to formation of peroxides, but to thermo-stable radiolytic compounds, which are more pronounced specially in fructose. Therefore, a careful control of all the conditions is necessary to

understand the products developed in irradiated sugars solutions and its effects on microbial growth, death and injury.

An interesting observation is that irradiated (using gamma Co^{60}, 20 kCi total activity at 1.389 Gy/s) powdered gelatins formed free radicals (0.31 to 1.62 x 10^{18}/g) which had a decay of about 50% after five (5) days (Bachman et al., 1974). The authors conducted microbiological studies (inoculation with spores of *Bacillus pumilus* E 601) and determined that at doses of 7.5 to 15 kGy the powdered gelatin was sterilized (from 3.4 - 4.25 $\log_{10}$ of initial population) and a post effect of irradiated samples was observed (after 2 and 6 weeks of irradiation initial numbers decreased). This suggests that higher doses are required to decrease 4.25 $\log_{10}$ of spores in powdered gelatin even with the formation of free radicals, which may have a post-treatment effect.

In a study conducted in the Texas A&M Food Engineering research laboratory, Castell-Perez et al. (2004), found that there were no significant changes in the quality of fresh-cut cantaloupes at irradiation doses below 1.5 kGy and below 1.0 kGy for the whole fruits. Higher doses (3.1 kGy) applied using a 10 MeV electron beam accelerator (single beam) caused undesirable effects on color, texture (firmness), size (density), sugar and carotene content, which were more noticeable in the whole fruits than in the packaged fresh-cut. Therefore irradiation at low doses was recommended as the optimum treatment for cantaloupes.

2.7. Gel structures

2.7.1 Physical properties of gel-based model food systems

Gelatins are the water-soluble products prepared by processes that involve the destruction of tertiary, secondary, and to some extent the primary structure of the native collagens (Ledward, 1986). A range of polysaccharides and polysaccharide mixtures are used as gelling agents by the food industry. Most of the gels are non-equilibrium states and the important factors in the gelation process are the polymer-solvent, polymer-polymer interactions and the effects of the preparative conditions on the extent and mechanism of phase (Morris, 1986). Polysaccharide gels may be classified as algal (alginates, agar, carrageenans), plant (pectins, starch), microbial (xanthan gum) and mixed (synergistic interactions of carob and guar gum or mannose-galactose).

In his review on gels, Morris (1986) cites that gelatin gels are quite soft and flexible, but their textural properties, in general, are very narrow whose strength of gelatin-based gels is dependent on the gelatin concentration, with little effect of ionic strength and pH. As a result, repulsive forces in the junction zones may reduce the formation of linkages between aggregated helices, leading to weakened gel structures, basic facts involving rheology are needed to understand the properties of many foods specially the called "soft solids", gels or gel-like.

From a rheological viewpoint a typical gel is a material that exhibits a yield stress, has viscoelastic properties and has a moderate modulus. From a structural point of view, a gel has a continuous matrix of interconnected material with much interstitial solvent (Fennema, 1996). The mechanical properties of various gels differ greatly. To

explain this, the behavior at large deformations should be considered. From a stress-strain curve several properties, as stress at fracture, can be determined. Fracture implies that the stressed specimen breaks, mostly into many pieces; if the material contains a large proportion of solvent, the space between the pieces may immediately become filled with solvent, rather than air. The modulus of the material (E), also called stiffness, is the ratio of stress to strain. For most gels, proportionality of stress and strain is only observed at very small strains, and at larger strains the quotient may be called an apparent modulus. The strength of the material is the stress (σ) at fracture (σ_{fr}). Terms like firmness, hardness, and strength are often used rather indiscriminately; sensory firmness or hardness often correlates with fracture stress. Modulus and fracture stress need not be closely correlated. These parameters also vary greatly with concentration of the gelling material. It is frequently observed that addition of inert particles ("fillers") to a gelling material increases the modulus but decreases the fracture stress. Part of the explanation of these divergences is that number and strength of the bonds in the gel predominantly determine a modulus, whereas fracture properties highly depend on large-scale in homogeneities (Fennema, 1996).

Many protein and polysaccharides have the ability to form gels which can hold a substantial amount of water and in some cases the water content of such gels may be as high as 99%. Protein gels are good model systems for the study of waterholding properties since the microstructure of the protein can be varied to give transparent gels containing much coarser aggregated structures. Factors such as pH, ionic strength, protein concentration, heating time and temperature as well as the kinetics of heating can

influence the microstructure of heat set protein gels (Hermansson, 1986).

When the amount of released water is measured water is either determined as water-holding capacity (*WHC*), which is the amount of water bound per gram of protein in dry matter or as the moisture loss (*ML*), which is the amount of juice released per gram of sample. The amount of water released will depend on the experimental conditions (Hermansson, 1986).

2.7.2 Quantifying gel properties

There are different methods to measure the strength of a gel, the most commonly used in the industry is called Bloom degrees, which is a puncture test performed with a Bloom Gelometer (Steffe, 1996).

A typical test is uniaxial compression, where the sample is squeezed between two plates with a moving plate (top) and a fixed plate at the bottom; the changes in the height (distance) and force are measured and related to strain (%) and stress (kPa). From the determination of the stress-strain relationship, the strain at fracture (ε_{fr}) may be called longness, but this term is rarely used. The terms shortness and brittleness are used, and they are closely related to 1/ ε_{fr}. The strain at fracture may vary widely; for gelatin ε_{fr} may be 3, and for some polysaccharide gels only 0.1. The ε_{fr} greatly depends on length and stiffness of the polymer chains between cross-links (Fennema, 1996).

Another parameter of significance is the toughness or work at fracture E_{fr}. This parameter is derived from the area under the curve from the relationship between strain and stress and expressed in joules per cubic meter. ($1J = 1kg \ m^2/s^2$, therefore $1J/m^3$, $= 1$ kg/ms^2), $1 \ N = 1 \ kgm/s^2$. This term explains how easy (or hard) is to break the sample

(brittleness) (Fennema, 1996).

All these parameters depend on time scale or rate of deformation, but in various manners. Several gels do not fracture at all when deformed slowly, but do so when deformation is rapid. In some cases, it is even difficult to distinguish between yielding and fracture. For some gels, fracture itself takes a long time (Fennema, 1996).

2.7.3 Effect of solutes on gel network

For an isoelectric gel the amount of water apparently unable to dissolve solutes is not a constant but depends upon their molecular size (Gary-bobo and Lindenberg, 1969). In addition, when the temperature is raised from 0.5°C to 23°C, the gels swell considerably. Therefore, the total water content increases from 4.65 to 9.0 gin/gin and at 38°C the gels are completely liquefied.

A large amount of the water in the gel is held by the disordered network of the gelatin chains. It is highly dependent upon temperature and pH. The structure of this region seems very open and does not offer any selectivity. However, it may be thought that for high enough gelatin concentration, this region would exercise a molecular sieving effect on large solutes, but this selectivity would be dependent on gelatin concentration (Gary-bobo and Lindenberg, 1969).

It is clear that the number of hydroxil (OH) groups (pH) of the solute is an important factor. The importance of this factor strongly suggests that the uneven distribution of nonelectrolytes in gelatin gel may very well reflect the partition ratio of these solutes between a "gel water phase" and the surrounding water phase.

2.7.4 Effects of irradiation on gel network

Most of the effects of irradiation on gelatin have been conducted at high doses and when the gelatin is in powder form. Bachman et al. (1974) state that the chemical reactivity of gelatin is determined by its molecular structure and its amino acid composition, which is also caused by components of non-collagenous origin such as sugars and impurities from the industrial production. The diversity of the functional groups makes it highly susceptible to modification.

Vieira and del Mastro (2002) subjected gelatin powder to gamma irradiation using a Co^{60} source (at a dose rate of 7 kGy/h 1.94 Gy/s) and to electron beam irradiation (dose rate of 11 kGy/s, or 0.011Gy/s) at doses of 0, 5, 10, 20 and 50 kGy. The authors found a significant decrease in viscosity depending on dose (0.5-0.7 cP at 5 kGy) and the higher the dose, the lower the viscosity. No differences were found between the radiation sources (i. e. dose rate) when measuring 10% aqueous solutions using a Brookfield viscometer at 40°C.

Bachman et al. (1974) reached different conclusions from studies of irradiated gels including the observation that gelatin is stable at low dose, viscosity and pH may decrease, and there is irreversible protein denaturation (due to descomposition in the amino group of glycol, and the imino group of praline and acetglycol, as the peptide bond in diketone piperazine yielding ammonia production).

In the study with gel preparations (10.8 to 13.7% water) subjected to irradiation doses of 10, 25 and 35 kGy using a Co^{60} source (1.389 Gy/s), Bachman et al. (1974) cite that the hydroxyproline content (a specific aminoacid of gelatin) does not undergo any

change as most of the aminoacids present; but there is an increase in the concentration of the carbonyl groups, viscosity increased when dose exceeded 25 kGy and the hardness of the gel decreased in doses between 5 kGy and 20 kGy. This study was conducted when gel was irradiated in powder form, and measurements were taken in gels formed with the irradiated samples. Therefore, irradiation may not have an effect on the nutrients present on a gel system.

2.8. Application of microscopy to the study of food systems

The observation of a single bacteria and its relative placement within a food system without using a microscope is practically impossible. With the advance of science different technologies have been developed to assist researchers in the study of microstructures at a macro scale.

2.8.1. Light microscopy

The three most common applications of microscopy to the study of food have been a) Scanning Electron Microscopy (SEM) and b) Transmission Electronic Microscopy (TEM), which allow magnifications from 20X to 100,000X, and c) Light Microscopy (LM), which gives a magnification range between 10 to 1,500X (Aguilera and Stanley, 1990).

Even when the magnification levels are limited LM has several advantages over its more potent counterparts: a) samples can be analyzed in real time (moving objects can be observed), b) there is no need of complicated sample preparation and processing of the Image display c) the environment where the sample is placed is versatile

(conditions can be adjusted as needed), and d) the use of different chemicals (staining methods) and fluorescence techniques have broadened the application of this technology to the explanation of microstructures (Aguilera and Stanley, 1990).

Light microscopy is the use of glass lenses, filters and light sources which help to visualize the specimen trough an eyepiece. The most common application is the bright field illumination, where light is transmitted from below trough a relative thin section or slice of the material and the image is formed and magnified above the sample and viewed by the observer. LM can also be enhanced by phase contrast or differential interference contrast optics in which the phase of the light is altered and then recombined to yield improved differentiation. Depending on the case phase contrast gives the best image while in others differential interference contrast is a superior technique. One method does not replace the other but they are complementary. Other modifications to this technology are polarizing microscopy where plane polarized light is allowed to impinge upon the specimen and fluorescence microscopy where a pair of filters are used to transmit only the desired wavelengths and combined with staining methods aloud color differentiation of macromolecules (Aguilera and Stanley, 1990).

2.8.2. Application of light microscopy (LM) to the study of bacteria motility

Most of the LM studies are conducted using electronic cameras to capture images. This application allows researchers to convert their observations to computer language, and to expand the techniques that can be used to describe the results of their studies.

Symanski et al. (1995) successfully applied LM to the study of the motility of

Campylobacter jejuni in carboxi metyl cellulose (CMC) to understand the movement of microbes depending on pH (5.0 to 8.5), viscosity of the gels (0.83 to 141.0 cP) and vibrational frequencies (20 to 30 Hz). The purpose of their study was to establish an *in vitro* model system for investigation of the interactions of *Campylobacter* with the epithelial cells of the gastrointestinal tract.

A better example of the understanding of permeability of gels and the use of LM is the study by Ruas-Madiedo and Zoon (2003) who determined the permeability of skim milk gels related to changes in temperature (20 to 30°C) and viscosity by measuring the movement of serum trough tubes and using the dyes rhodamine and acridine orange to differentiate the chemical compounds of the gels from lactic acid bacteria (*Lactococcus lactis*). With the use of dyes and LM they could not only made bacteria counts (numbers) but to describe the bacteria distribution in the milk gels (homogeneity in distribution or cluster formations) and also the gel structure.

The motility of the strain *Escherichia coli* K-12 MG1655 has been described by researchers of the *Escherichia coli* genome Project at the University of Wisconsin (Liu et al. 2005). As part of their studies on the carbon source utilization they have recorded the strain motility in fluids (glucose, glycerol, succinate, alanine, acetate and proline). Plenty of information regarding to this strain (growth and genome sequencing) is available from this group (Liu et al. 2005).

CHAPTER III

MATERIALS AND METHODS

3.1 Cultures

3.1.1 Acquisition

Lyophilized cultures of non-pathogenic strains, previously selected because of their similar growth characteristics to the three most common food-borne pathogens (*E. coli* O157:H7, *L. monocytogenes* and *Salmonella sp.*) were obtained from different sources. *E. coli* K-12 MG 1655 was acquired from Dr. M. Berlyn (*E. coli* Genetic Stock® Center, University of Wisconsin), *Salmonella* LT2 from Dr. Larry Beuchat (University of Georgia), *Enterobacter aerogenes* B199A from Dr. Don Schaffner (Rutgers University), and *L. innocua* NRRL-B 33003 and NRRL-B 33314 from the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) Culture Collection (Peoria, IL). The pathogenic strains (*E. coli* O157:H7 933, *L. monocytogenes* ATCC 51414, and *Salmonella* Poona) were obtained from Dr. A. Castillo and Dr. G. Acuff from the Food Microbiology Laboratory (Animal Science Department, Texas A & M University). The vials were maintained in a Harris freezer (Scimetric, Inc., Missouri City, TX) at -80ºC until further use.

3.1.2 Media preparation

Tryptic Soy Broth (TSB) was prepared in tubes (10 ml), and Tryptic Soy Agar (TSA) in glass beakers (approximately 2L), and transferred to sterilized disposable

plastic Petri dishes, 100 x 15mm (Fisher Scientific, Canada) after sterilization. Both media cultures were obtained from DIFCO^{TM} (MD, USA).

Tryptone or trypticase (17.0 g), phytone or soytone (3.0 g), sodium chloride (5.0g), dipotassium phosphate (2.5 g) and dextrose (2.5 g) were weighted in a Sartorius analytic balance (Sartorius GMBH, Göttngen, Germany) and mixed in a Thermolyne multistirrer plate "4" (Sybron/Thermoline, Iowa, USA), adjusted to pH 7.3 by addition of chloridric acid (HCl) or 1M sodium hydroxide (NaOH). The pH was measured with a potentiometer Accumet model 25 (Denver Instrument Company; CO, USA). The ingredients were dissolved in distilled water (1.0 L), warmed slightly to complete solution, and adjusted to pH 7.3 ± 0.2 by addition of 1 M sodium hidroxide (NaOH) or hidrochlorydric acid (HCl). The final solution was dispensed into tubes (9ml), and sterilized by autoclaving 15 minutes at 121°C using a Tuttnauer Brinkmann 2540E autoclave (Jerusalem, Israel).

TSA was prepared by weighing and mixing trypticase or tryptone (15.0 g), phytone or soytone (5.0 g), sodium chloride (5.0 g) and agar (15.0 g). The ingredients were suspended in 1.0 L of distilled water, mixed thoroughly, heated with frequent agitation, and boiled for about 1 minute to dissolve completely. The pH was adjusted to 7.3 ± 0.2. The boiled mixture was autoclaved 15 minutes at 121ºC, then cooled in a water bath to 45ºC and poured into sterile Petri dishes. The plates were left to solidify at room temperature and stored at 4ºC in a Frigitemp® Controlled Environmental Room (New York, USA) until further use.

Eosin Methylene Blue Agar (EMB), Modified Oxford Media (MOX), and Xilose

Lisine Desoxycholate Agar Base (XLD) (DIFCO^{TM}, MD, USA) were prepared as selective media for *E. coli*, *Listeria* and *Salmonella* species following the same procedure as TSA (following manufacturer's instructions) with the exception that XLD does not require boiling before autoclaving.

3.1.3 Preservation and recovery

Either one loop or the lyophilized pill of each of the original cultures was recovered in the tubes containing TSB under a Laminar Flow Work Station (Forma Scientific, Inc., Ohio, USA), and grown in an Equatherm incubator (Curtin Matheson Scientific, Inc., TX, USA) for 24 hours at 37ºC. The cultures were then transferred into Protect^{TM} Bacterial System plastic vials (Key Scientific, TX, USA) containing glycerol as 'cryopreservative solution', and sterile chemically treated porous plastic beads. The vials were maintained in a Harris freezer (Scimetric, Inc., TX, USA) at -80ºC until further use.

Before each irradiation treatment, one plastic bead containing the desired culture was aseptically transferred into a tube containing 10ml of TSB, and incubated at 37ºC for 24 hours. All the aseptic work was made under a Laminar Flow Work Station. To facilitate recovery, tube slants containing TSA were inoculated and grown at 37ºC for 24 hours and kept in a Frigitemp$^{®}$ Controlled Environmental Room (New York, USA) at 4ºC for a maximum period of 30 days until further testing.

3.2 Preliminary studies

3.2.1 Surrogate/indicator selection

A drop of the recovered cultures (0.1 ml) containing a population between 10^7 and 10^9 CFU/ml was pipetted to the surface of gelatin-based cylinders (Figure 3.1b).

Approximately 2.0 ml (10% w/v) of gelatin was made into cylinders by mixing 100 g of powdered collagen, commercial unflavored gelatin (The Kroger Co., Cincinnati, Ohio), with 1.0 L of distilled water at room temperature (20°C). The mixture was heated and stirred using a Thermolyne Multistir Plate "4" (Sybron/Thermoline, Iowa, USA) until totally dissolved. The mixture was then cooled in a Aquabath water bath (Lab-Line Instruments, IL, USA) to 45°C to adjust to pH 7.0 by addition of chloridric acid (HCl) or 0.5 M sodium hydroxide (NaOH). The mixture was heated again until its boiling point to ensure sterilization and to avoid foaming.

Approximately 60 ml of the sterile gelatin were transferred with sterile pipettes to sterilized disposable plastic Petri dishes. A line (120° angle clockwise from the vertical line) was used in the Petri dishes (100 x 15 mm) as a guide for the positioning of the cylinders (A, B, C, where B was the center), which were placed 2 cm apart (Figure 3.1a and b).

This design was developed as a preliminary study to determine the optimum gelatin concentration, sample volume and aseptic techniques to produce a gel-based system that could be inoculated without affecting the surrogates and in sufficient amount to allow dilutions and counts of the initial inoculum and irradiated samples.
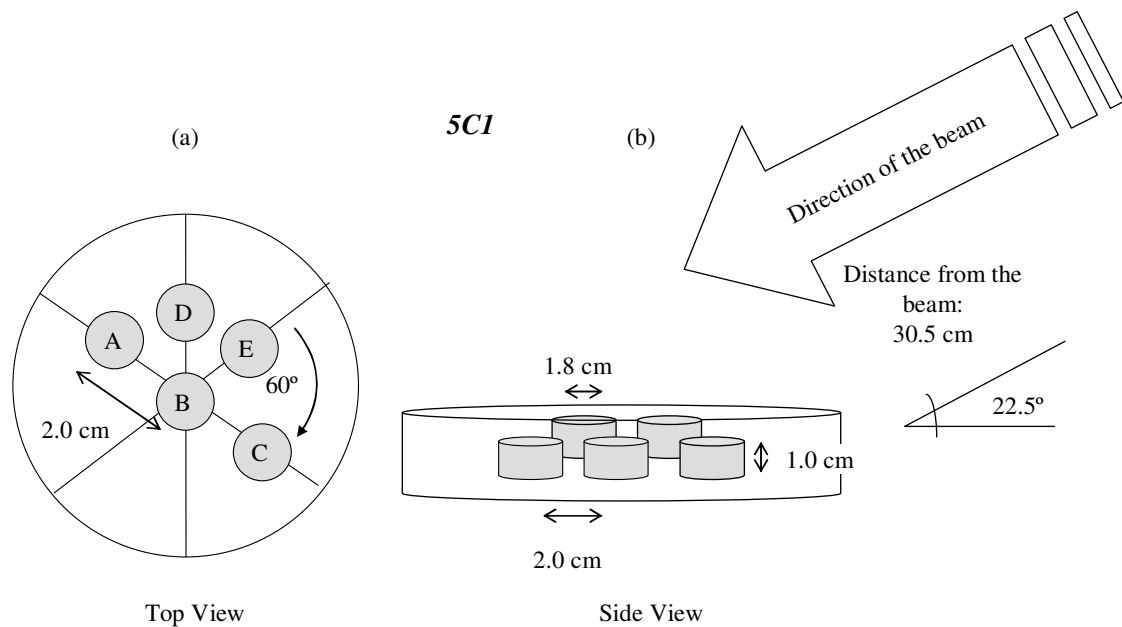
Figure 3.1. Placement of inoculated gel cylinders in Petri dishes for irradiation tests using a 2.0 MeV Van de Graaff linear accelerator at room temperature (20°C). (a) Top view, (b) Side View.

5C1: 5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit.

Some of the assumptions for this design were that (1) all the microbes remained at the surface of the gels and, that (2) dose penetration was uniform within the cylinders. The positioning of the gel cylinders in the Van De Graff electron beam accelerator was based on the fact that at these points the minimum variation (1.2%) in the readings of absorbed dose was observed. Dose was measured with a Farmer Dosimeter (J. L. and associates, Glendale, CA), using an empty Petri dish covered with two layers of Ziploc® bags (Johnson & Son, Inc, Racine, WI) in the six possible positions at 2cm around the center (60º angle beginning from the vertical axis). It is important to note that each plastic layer caused a decrease in dose of 5.0 Gy when the plate was placed 22.5 degrees from the source of the beam (Figure 3.1b).

The Petri dishes were then covered with a lid and placed in a flat surface inside a Frigitemp® Controlled Environmental Room at 4°C overnight. Surface flatness was measured with a level at each Petri dish across both axes. The gels were perforated with a sterile sharp corer (0.018m diameter) in the three marked points (A, B, C) and the remaining gel was removed to make cylinders (volume of 2.0 ml). All the above procedures were made following aseptic techniques. The gel cylinders were positioned as indicated in Figure 3.1a.

The prepared Petri dishes were sealed with petrifilm (Parafilm American National Corp., Menasha, WI) around the edges, double packed in ZipLoc® bags and packed in a cooler (15ºC) for future handling and storage between irradiation treatments.

3.2.1.1 Irradiation treatments

Inoculated gel cylinders (as described in Section 3.2.1) were subjected to five irradiation doses (0 to 1.0 kGy in increments of 0.2 kGy), in three repetitions (A, B, and C), using a 2.0 MeV Van de Graaff Electron Accelerator (High Voltage Engineering Cooperation) located at Texas A&M University. Absorbed dose was determined from the count of $Cx10^{-8}$ coulombs in the Van de Graff and related with dose previously determined with a Farmer Dosimeter as described in Section 3.2. The time for dose application was recorded in seconds using a stopwatch (Fisher Scientific, Canada). Dose rate was kept constant (around 0.5 –1.5 Gy/s) by controlling the cathode temperature (300 µA) of the Van De Graff. Temperature and percent relative humidity (%RH) were recorded using a Heavy Duty Hygro-Thermometer Model 407445 (Extech Instruments Corporation, MA, USA) with an accuracy of 0.1ºC and 0.1% RH.

After irradiation, the 2.0 ml gel cylinders were transferred aseptically into sterile glass tubes. The tubes were placed in the Equatherm incubator at 37ºC for 15 minutes, until melted, and mixed in a vortex (Vortex Genie2, Scientific Industries, N. Y., USA). One (1.0) ml of the homogenized melted gel system was distributed in four Petri dishes containing TSA and counted as dilution zero. One (1.0) ml was diluted subsequently in tubes containing 9 ml of peptone water (0.1%) for a fold of nine dilutions (10^0 to 10^7), from which 0.1 ml of each dilution was distributed with a sterile glass rod in the TSA. All the plates were placed in the incubator at 37ºC for 24 hours. Viable colonies of bacteria (CFU/ml) were counted using the Plate Count Agar (PCA) technique for all the dilutions following the method by Busta et al. (1984).

3.2.1.2 Calculation of radiation D_{10} *values*

Radiation D_{10} values were calculated from the negative inverse slope of the logarithm of viable population (CFU/ml) versus dose (kGy) as indicated in Figure 2.1. The REG procedure from the SAS® System (Release 8.01, SAS Institute, Inc. NC, USA) was used for statistical analysis. The reaction rate constant (k) was calculated based on Eq. (2.6) by calculating the negative of the slope of the linear regression between the natural logarithm of the fraction of survivors (ln N/No) and dose (kGy) using Microsoft® Excel, 2002 (Microsoft Corporation, WA).

The radiation D_{10} values (kGy) for each non-pathogenic strain were compared with those obtained for each pathogenic strain within species by using analysis of covariance. The hypothesis was that if the proposed non-pathogenic strains had similar or higher radiation D_{10} values to those for the pathogenic strains they could be used as surrogates of food-borne pathogens under electron beam irradiation. In addition the non-pathogenic strains could also be equally or more resistant to irradiation doses at the range of this study (0 to 1.0 kGy) at the established conditions (sample size and dimensions, chemical composition, dose ranges, temperature, irradiation source and inoculum size). The strain that showed similar or higher resistance to irradiation than the studied pathogenic species was selected as the most suitable surrogate.

Due to the variability in microbial population count measurements, the population that survived the irradiation treatment (S) was mathematically adjusted in reference to the numbers of the inoculum (A) for purpose of comparison between treatments. For example, if the population inoculated (A) was 7.0 $\log_{10}$ CFU/ml and the

survivors (S) at 1.0 kGy were 5.5 $\log_{10}$ CFU/ml, A was assumed to be 0.0 $\log_{10}$ CFU/ml and S to be -1.5 $\log_{10}$ CFU/ml. These calculations simplified the analysis of the results in the damaged population (negative values or reductions from the undamaged population) without affecting the trends (slope) and therefore the radiation D_{10} values, compared with percentages or ratios (S/A). The same concept was applied for the calculations of recovery.

3.2.2 Model food systems

3.2.2.1 Basic composition

Different levels of the three most important sugars (glucose, fructose and sucrose) were prepared to mimic the different ranges in composition and maturity levels in melons, maintaining a pH (7.0 ± 0.3) and water activity (0.95 ± 0.01) that allows microbial growth (Table 3.1), The initial basis was a combination of several studies (Sharaf et al., 1989; Agravante et al., 1990; Wang, 1994; Villanueva et al., 2004; USDA, 2004; see Appendix C for details). Crystallized Fructose, and powdered glucose and sucrose were obtained from Fisher Scientific (Fisher Scientific Co., NJ, USA). The sugars were added to unflavored gelatin in three different amounts (%) at three ratios (fructose : glucose : sucrose) to simulate three ripening stages of melons (Table 3.1).

Variations were prepared by weighting separately the sugars from the gelatin to avoid Maillard reaction. A Sartorius analytical balance (GMBH; Göttngen, Germany) and a stirrer (Sybron/Thermoline; Dubuque, Iowa) were used to dissolve the gelatin in half of the distilled water, and the sugars in the other half (volumes varied depending of

Table 3.1

Sugar content and ratios used to mimic the different maturity levels in melons

Maturity levels	% of total weight in sugars	Ratio of sugars (Fructose:Glucose:Sucrose)
Early Ripe (*ER*)	3.0%	1.5:1.5:0
Moderately Ripe (*MR*)	5.5%	1:1:1
Ripe (*R*)	8.0%	0.5:0.5:2.0

Sources: Sharaf et al. (1989), Agravante et al. (1990), Wang (1994), Villanueva et al. (2004), and USDA (2004). See Appendix C for details.

the amount prepared). The pH was adjusted to 7.0 by addition of HCl or 1M NaOH, the solutions were boiled and cooled in a water bath to 45ºC before mixing. The liquid mixtures were added at a constant volume (20 ml) with a sterile pipette to sterilized disposable plastic Petri dishes (100 x 15 mm). Samples were prepared in triplicates and non-sugars systems (*C*) or controls were evaluated for all the irradiation treatments.

The rationale behind this procedure was that fructose and glucose levels significantly decrease during the ripening of fruits such as cantaloupes and melons, while sucrose increases (Tucker, 1993). Therefore, a evaluation of effects of different sugar ratios in media (gel) for bacteria growth should be useful for the optimization of the irradiation treatment of fruits.

3.2.2.2 Harvesting method

Bacteria were inoculated and recovered from gelatin cylinders prepared and harvested as in Section 3.2.1.1 and compared with those harvested by using the standard stomacher procedure. The purpose of this study was not to evaluate bacteria but the harvesting method. The melting procedure allows counting of bacteria without addition of distilled water (dilutions), therefore sterilization can be measured by platting the sample directly in agar for counting. To compare with a different gel system, plain agar cylinders were prepared by mixing 15g per liter of DIFCO® agar (Beckton Dickinson and Co., MD), boiling, autoclaving (121ºC for 15 minutes), transferred to sterile petri dishes and left overnight at 4ºC.

The inoculated systems (0.1ml) were left in the Frigitemp® Controlled Environmental Room at 7ºC for 24h. Three (3) gelatin cylinders were melted in the

Equatherm incubator at 37ºC. Three (3) gelatin cylinders and three (3) agar cylinders were blended in a Stomacher with 18ml of sterile distilled for 120s at High Speed. Agar was not melted because of its higher melting temperature. The treatments were serially diluted, platted in TSA, incubated and counted as in Section 3.2.1. Recovered populations (CFU/ml) were compared to determine the optimum harvest method.

3.2.3 Irradiation treatments

3.2.3.1 Penetration depth

A study was conducted to determine the effect of the model system cylinders height; thus, the uniformity of applied dose and electron beam penetration depth, on the killing of bacteria.

For this study, the inoculum (0.1 ml) was added to the gelatin and mixed before cooling overnight to assure uniform distribution of bacteria within the gel. The ratio used was 5.0 ml of previously recovered inoculum (*Escherichia coli* K-12 MG1655) per liter of gelatin. The samples were positioned as in Section 3.2.1 (Figure 3.1) using configuration *5C1*, and sliced in four layers of 0.25 cm height (*T*, *MT*, *MB*, *B*) and melted as in Section 3.3.3 (Figure 3.2) after irradiation at 1.0 kGy. Microbial populations (CFU/ml) were counted at each of the four layers of gelatin. A control sample (non irradiated) was also prepared and measured under the same conditions (room temperature 20˚C).

3.2.3.2 Sample dimensions and placement

Due to insufficient information about the optimum positioning of the samples and the dose delivered by the beam, different sample sizes and positioning were evaluated to increase the accuracy of the experiments and to account for the variability in doses achieved within the gel containing plate. Plain gelatin (10% w/v) was prepared as in Section 3.2. Then, three setups were tested: (a) Five (5) cylinders (*5C1*) (1.8 cm diameter x 1.0 cm high to obtain approximately 2 ml of sample), were placed in a Petri dish positioned at a 22.5° angle and 30.5 cm from the beam (Figure 3.1); (b) one (1) cylinder (the diameter of a standard Petri dish: 8.5 cm diameter) placed at the center with variation in thickness: 1.0 cm (*1C1*), 0.5 cm (*1C5*) and 0.25cm (*1C25*), all these treatments were also placed at a 22.5° angle and 30.5 cm from the source of the beam (Figure 3.3); (c) one (1) cylinder (*1C25M*) with the smaller thickness (0.25 cm) and a smaller diameter (6.0 cm) was irradiated at one-half of the distance (15.25 cm) from the beam and at a perpendicular position (67.5° angle) from the beam source (Figure 3.3).

3.2.3.3 Dose ranges

All the gels were subjected to doses up to 5.0 kGy (0.5, 1.0, 2.5 and 5.0 kGy). The gels positioned in the optimum design (*1C25M*), which was determined from Section 3.2.3.2, were subjected to doses up to 1.2 kGy at increments of 0.3 kGy. This setup allowed the collection of enough measurements of survivors for adequate linear regression analysis. Absorbed dose was determined as in Section 3.2.1.1. Doses higher than 1.2 kGy in the *1C25M* configuration resulted in complete killing of the inoculated
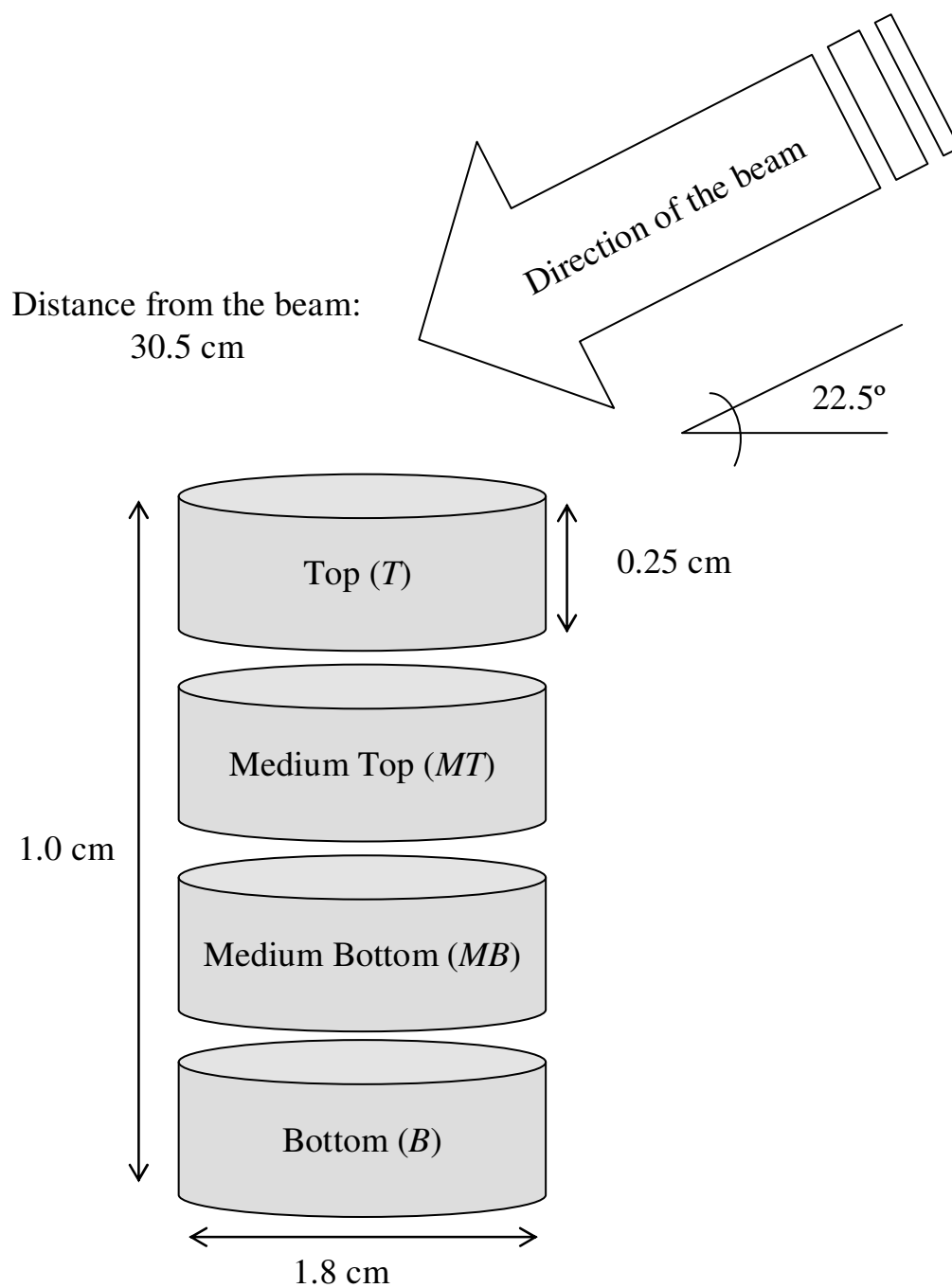
Figure 3.2. Gel system dimensions and placement for evaluation of penetration depth of the electron beam (*T* means top layer, while *B* means bottom layer of the cylinder).
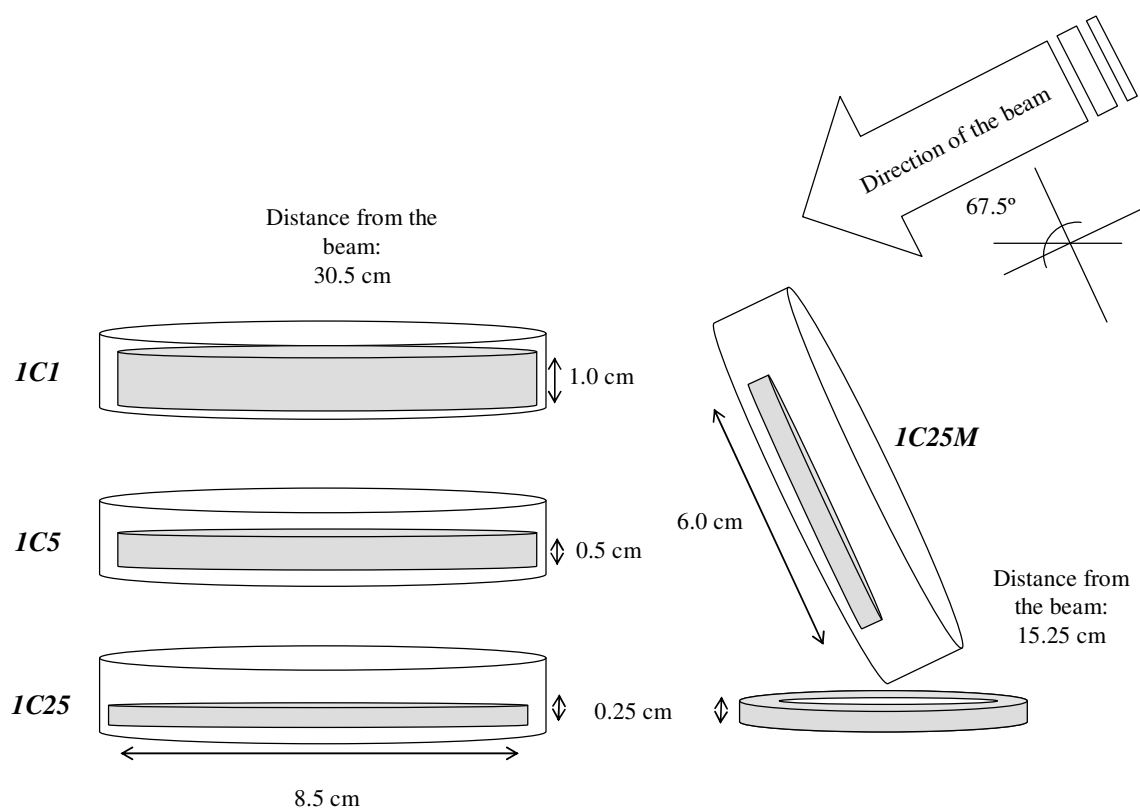
Figure 3.3. Placement of inoculated gel cylinders in Petri dishes (100 x 15 mm) for irradiation tests using a 2.0 MeV Van de Graaff linear accelerator at room temperature (20°C). (a) Top view, (b) Side View.

1C1 = 1 cylinder - 1.0 cm height and 8.0 cm diameter, 22.5°, 30.5 cm from beam exit, *1C5* = 1 cylinder - 0.5 cm height height and 8.0 cm diameter, 22.5°, 30.5 cm from beam exit, *1C25* = 1 cylinder - 0.25 cm height height and 8.0 cm diameter, 22.5°, 30.5 cm from beam exit and *1C25M*: = 1 cylinder – 0.25 cm height height and 6.0 cm diameter, 67.5°, 15.25 cm from beam exit.

population (*A*), which limits the amount of information (at least five points where dose and population can be counted) that can be used to perform a linear regression.

3.2.3.4 Microbial analysis

Prior to irradiation, the five gel cylinders (*5C1* configuration, (a) in Section 3.2.3.2) were inoculated with 0.1ml of the selected surrogate (*Escherichia coli* K-12 MG1655). The cylinders with a configuration covering the whole plate (*1C1*, *1C5*, *1C25* and *1C25M*, (b) in Section 3.2.3.2) were inoculated with 0.5 ml of the surrogate. *E. coli* K-12 MG1655 was recovered as in Section 3.1.3. The inoculated systems were maintained at 4°C between irradiation and harvesting.

After irradiation in the Van de Graaff accelerator the gel cylinders (*5C1*) were aseptically transferred to sterile glass tubes. These tubes were placed in the Equatherm incubator at 37ºC for 10 minutes until melted, and mixed in a vortex. One (1.0) ml of the homogenized melted system was distributed in four plates containing TSA and counted as dilution zero, and 1.0 ml was diluted nine times subsequently in tubes containing 9.0 ml of peptone water (0.1%) (1 to 10^7), from which 0.1ml of each dilution was distributed with a sterile glass rod in TSA and grown in an Equatherm incubator at 37ºC for 24 hours. Viable colonies (CFU/ml) were counted following the standard plate count method.

The gel cylinders of configuration (b) in Section 3.2.3.2 (*1C1*, *1C5* and *1C25*) were melted in their original Petri dish, while the 6.0 cm diameter cylinder (*1C25M*) was irradiated when it was covering the whole plate (8.5 cm diameter) and aseptically bored (reduced to a diameter of 6.0 cm) and transferred to a sterile plate to avoid migration of

the microbes from the edges. The same "modified harvesting method" was followed in all the experiments where this configuration (*1C25M*) was used.

Radiation D_{10} values and reaction rate constants (*k*) were calculated as indicated in Section 3.2.1.2.

3.2.4 Recovery and maintenance

3.2.4.1 Maintenance at low temperatures

Using the *5C1* design, (a) in Section 3.2.3.2, and conducting simultaneously the determination of D_{10} values in described in Section 3.2.3.1, the four gel systems (*C*, *ER*, *MR* and *R*) were prepared in duplicate as in Section 3.2.2.1. The systems were subjected to irradiation at 1.0 kGy using the same procedures described in Section 3.2.1.1 and maintained in their sealed package at 4°C in a Frigitemp® Controlled Environmental Room (New York, USA). The irradiated (*I*) gels (*CI*, *ERI*, *MRI* and *RI*) and non-irradiated controls (*C*, *ER*, *MR* and *R*) were melted every 24 hours for 4 days. Microbial populations were counted as described in Section 3.2. One (1.0) kGy was used to ensure the ideal remaining population for recovery studies, around 10^2-10^3 CFU/ml (Legan and Vandeven, 2000).

3.2.4.2 Storage at different temperatures

The effect of storage temperature in the ability of the population to survive or recover decreased populations was evaluated using the medium ripe (*MR*) samples in the *5C1* configuration. This experiment was selected so that the variability within samples

per treatment could be assesed. The samples were subjected to irradiation (1.0 kGy) using the same procedures as before (Section 3.2.1.1), and maintained in their sealed packages at three (3) temperatures (4°C, 10°C and 20°C) and melted every 24 hours during 68 hours for dilutions, platting and counts after 24 hours.

3.3 Kinetics of survival and recovery under irradiation treatments

3.3.1 Effect of maturity stage on radiation D_{10} *values*

Using the modified configuration (*1C25M*) described in Section 3.2.3.2, survival curves for the samples mimicking the different maturity levels (*ER*, *MR* and *R*) were obtained by plotting irradiation dose (kGy) and viable population (CFU/ml) for systems prepared as described in Section 3.2.2.1. The radiation D_{10} values were calculated using the standard procedure (Section 3.2.1.2) and the results were compared to evaluate the effect of fruit maturity levels (sugar content) on the killing efficiency of the irradiation treatment. The reaction rate constant was also calculated as in Section 3.2.1.2.

3.3.2 Kinetics of recovery

Gel-based systems were prepared with different sugar contents (*C*, *ER*, *MR* and *R*) inoculated with 0.5 ml of the selected surrogate (*E. coli* K-12 MG1655) and irradiated in the modified configuration, *1C25M* (Section 3.2.3.2). After irradiation at 1.0 kGy the gel cylinders were aseptically extracted, transferred to sterile Petri dishes and sealed for preservation at 20°C. The modified harvesting method (Section 3.2.3.4) was followed after irradiation maintained the samples after irradiation in a container sealed with

parafilm and in a ZipLoc® bag. Microbial counts were made every 24 hours for 60 hours.

The viable population (CFU/ml) means for each dose treatment were calculated after irradiation and used as the initial surrogate population (inoculum size in CFU/ml). Growth curves were developed by plotting time in hours (x axis) versus population growth in $\log_{10}$ of CFU/ml (y axis). From this relationship the length of the lag phase (L) was calculated from time 0 to the beginning of maximum growth (the lower asymptotic $\log_{10}$ bacterial count as time in hours decreases indefinitely) as in Figure 2.1. Generation time (GT) in hours was obtained from the relationship between time (t) and population (N, in CFU/ml) at the inflexion points of the growth curves (points B and C in Figure 2.1) using Eq. (2.17). Maximum population (CFU/ml) was determined as viable populations at the upper asymptotic $\log_{10}$ bacterial count as time (t) in hours increases indefinitely as indicated in Section 2.4.1.

3.4 Validation studies

3.4.1 Irradiation treatments

3.4.1.1 Surrogate validation in the modified configuration

The modified configuration, *1C25M* (Section 3.2.3.1), was used to irradiate the pathogens (*E. coli* O157:H7 933, *L. monocytogenes* ATCC 51414, and *Salmonella* Poona) in 10% gels (no-sugars) prepared and harvested as described in Section 3.2.3.2. Applied irradiation doses ranged between 0.3 and 1.2 kGy (in increments of 0.3 kGy) using the 2 MeV Van De Graaff accelerator. Dose rate was kept constant between 4.3 and 6.0 Gy/s. Irradiation experiments were carried out as described in Section 3.2.3.1

and radiation D_{10} values and reaction rate constants (k) were calculated as indicated in Section 3.2.1.2.

3.4.1.2 Modified food (gel systems) versus real cantaloupes

The suitability of using the *5C1* and *1C25M* configurations was validated using real cantaloupes. One fresh cantaloupe was acquired in a local store (HEB, TX) one day before treatment and stored at 4°C. One (1) hour before irradiation the fruit was rinsed with soap and aseptically peeled. Slices were extracted from the pulp, bored and aseptically placed in Petri dishes in the same arrangement as the 5C1 configuration of the gel cylinders (Figure 3.3). The *5C1* shaped cantaloupes were inoculated with 0.1 ml of the recovered surrogate (*E. coli* K-12 MG 1655) and subjected to irradiation (1.0 kGy). The *1C25M* cantaloupe slices were inoculated with 0.5 ml of the surrogate and the most resistant pathogen (*Listeria monocytogenes* ATCC 51414) and subjected to doses up to 1.2 kGy (in increments of 0.3 kGy). The Petri dishes were packed and transported as described in Section 3.2.1. The irradiated cantaloupes were stomached at high speed for 120 seconds and serially diluted for platting. Microbial counts were made after incubation for 24 hours in TSA at 37°C. D_{10} values and reaction rate constants (k) were calculated as described in Section 3.2.1.2.

3.4.2 Physical properties of the model food systems

Texture analysis of the gel-based systems was conducted using the five cylinders configuration (*5C1*). The different gel treatments (*C*, *ER*, *MR*, *R*) were stored at 4°C. This study was also conducted in parallel with the preservation of bacteria at different

temperatures (Section 3.2.4.2) with *MR* systems stored at 10°C and 20°C. The samples were subjected to uniaxial compression using a TA.XT2 Texture Analyzer connected to a computer with the software Texture Expert Version 1.16 (Stable Microsystems Texture Technologies, Co. New York, USA). Samples were allowed to equilibrate at room temperature (20°C) for 15 minutes prior measurements. The gels were compressed until fracture (7 mm height) at 0.5 mm/s with a force of 0.05 N. Force (in N) and deformation at fracture (in mm) were used to calculate stress at fracture (σ_{fr}), strain at fracture (ε_{fr}), and elastic (or Young's) modulus (E), where σ_{fr} is the maximum force achieved before fracture, in kPa; ε_{fr}, the maximum strain when the sample was destroyed, in %; and E is the slope of the linear relationship between stress and strain (up to 45 %).

Density (kg/m^3) of the gels was determined by weighing the cylinders (in grams) using an analytical balance, and measuring the volume (in ml) displaced from a filled beaker with a pipette at room temperature. The pH of the systems was previously adjusted to 7.0 ± 0.3 as explained in Section 3.2.1. The time for the gels to melt at 37°C was recorded.

3.4.3 Bacteria distribution in the gel systems

3.4.3.1 Microbial analysis

The motility of the surrogate (*E. coli* K-12 MG1655) within the gel systems was measured by placing 0.1 ml of recovered culture in gelatin and agar cylinders using the *5C1* configuration. Gelatin and agar cylinders were prepared as in Section 3.2.2. Eighteen (18) inoculated cylinders were left covered in sterile Petri dishes at room

temperature (20ºC) for three hours. The cylinders were divided through the horizontal axis into four slices (0.25 cm thick) (Figure 3.4). Each slice (*T*, *MT*, *MB* and *B*) was melted separately in sterile tubes (gelatin systems) or stomached for 120 seconds at high speed (agar systems), and diluted for platting in three repetitions every 30 minutes for three hours. Microbial populations were counted in TSA as in the previous experiments. The rationale for this experiment was to determine whether the bacteria migrated to the inside of the gel systems in the time period between inoculation, irradiation and plate counting.

This information is important because of the short penetration depth of the 2.0 MeV Van De Graaff linear accelerator (0.25 cm in water) and the understanding that "surface" inoculation is a relative term in porous or gel-like systems.

3.4.1.2 Microscopy analysis

The displacement of bacteria through the surface (horizontal, *x* and *y* axis) and depth (vertical, *z* axis) of the gelatin systems was assessed using microscopy techniques. Gelatin systems without sugars (*C*) were prepared in Petri dishes as in Section 3.2.1 and left to solidify overnight at 4°C prior to the experiments.

On the day of measurement, the prepared gelatin systems were melted at 40°C and transferred to sterile Petri dishes containing a glass slide until it reached a thickness of approximately 1.0 mm. The covered slides were left to solidify at 4°C. After three hours the glass slides were inoculated with 0.5ml of previously recovered *Escherichia coli* K-12 MG1655 to reproduce the same ratio used in the gel systems (0.04 ml/cm^2) and a coverslip (22 x 22 mm) from Baxter Health Science Pro Care Corp (IL) was
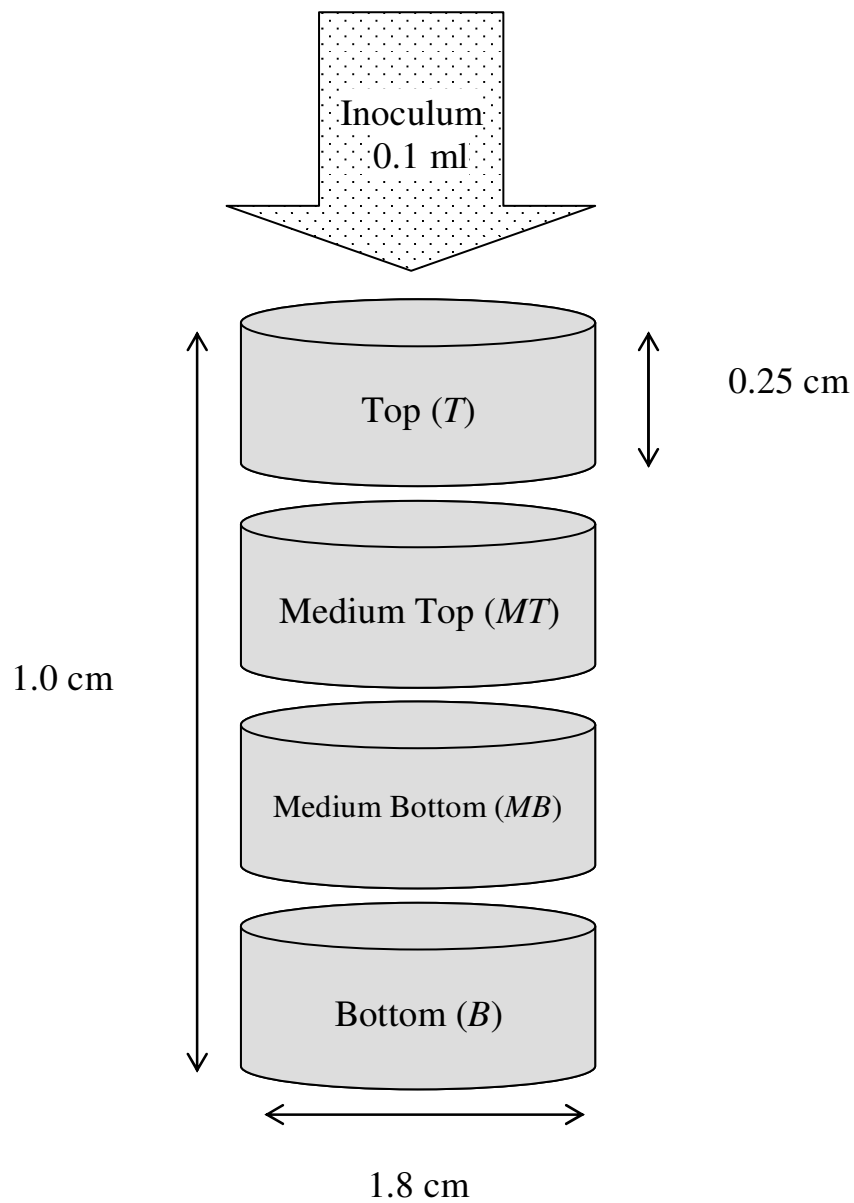
Figure 3.4. Sample design for evaluation of the motility of *Escherichia coli* K-12 MG1655 in gelatin and agar systems using standard plate count techniques.

The cylinder shaped system shown above is divided horizontally into four slices (0.25 cm thick).

placed on the top every hour (Figure 3.5a). The samples were analyzed in a Phase

Contrast Microscope (Axiophot from Zeizz W. Germany) and pictures taken with a

Nikon DXM 1200 Digital Camera at a frame rate of 13.5 frames per second using a 20X

magnification lens. The pictures were then processed using the software Meta Vue, Meta

Imaging Series from Universal Imaging Corporation (USA). This setup allowed the

study of the surface (x axis) motility of the bacteria in the gel-based systems.

The Java application ImageJ. 1.33u from the National Institute of Health (USA)

was used to measure the position of 8 bacteria in the two axes (x and y) in pixels into an

area of 22,500 μm^2 (150 x 150 μm). Two (2) bacteria were identified on each quarter

(quadrant) of the pictures following a cardinal position (Figure 3.5b).

Bacteria density (ρ_b) in Units/μm^2, was calculated by counting the numbers of bacteria in

the same area. Swimmers (S_w) were also counted.

Positions (x, y) in pixels were converted to μm using a scale control. The

displacement (d) was calculated as the hypotenuse between the horizontal and vertical

movement using (Eq. 3.1) (Figure 3.5c),

$$c^2 = a^2 + b^2$$
$$d^2 = (x_1 - x_2)^2 + (y_1 - y_2)^2$$

(3.1)

The velocity (v) in $\mu m/s$ was calculated using (Eq. 3.2).

$$v = \frac{d}{t_2 - t_1}$$

(3.2)
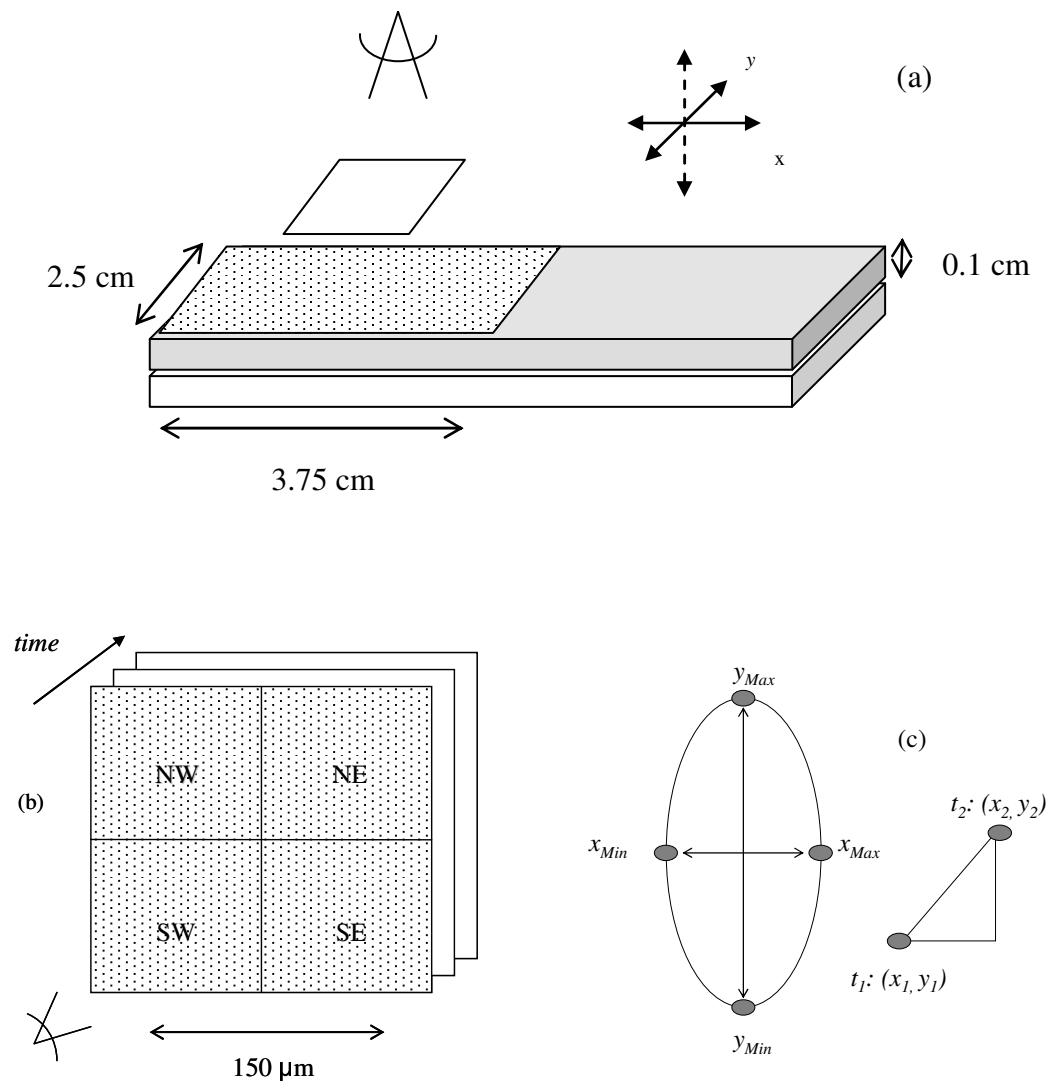
Figure 3.5 Handling of gelatin samples for evaluation of horizontal displacement of *Escherichia coli* K-12 MG1655 using light microscopy. (a) placement of a layer of gel on a microscope slide and inoculation; (b) digital picture sampling division for tracking bacteria; (c) nomenclature used to calculate movement based on positioning of bacteria.

where d is the distance of displacement (µm) and t_2 and t_1 the time interval (s).

The area of bacteria movement was calculated by the average of the diameters of the two axis (D_x and D_y) as,

$$\overline{D} = \frac{D_y + D_x}{2}$$
$$\overline{D} = \frac{(y_{Max} - y_{Min}) + (x_{Max} - x_{Min})}{2}$$

(3.3)

Where y_{max} and y_{min} are the maximum and minimum values in the vertical axis measured in pixel units, and x_{max} and x_{min} are the maximum and minimum values for the horizontal axis.

And finally, the area of movement, in µm^2, was calculated as,

$$\overline{A} = \frac{\pi \overline{D}^2}{4}$$

(3.4)

All the calculations were made using the Software Microsoft® Excel 2003 (Microsoft Co., WA) and the outputs were processed using Plot-It for Windows Version 3.2 from Scientific Programming Enterprises, MI.

Petri dishes containing previously formed gels (0.4 cm high) were inoculated with *Escherichia coli* K-12 MG1655 with 1.5 ml of recovered cultures and left at room temperature (20°C) during the period of study. Bores of the center of the gels were taken every hour during four hours and a gel mixed with bacteria was prepared as control (Figure 3.6a). This study was developed to evaluate the vertical motility of the bacteria in the gelatin through the height of the gelatin cylinders or from surface to bottom.

The bores were observed upside down in the Phase Contrast Microscope (Axiophot from Zeizz W. Germany) to achieve more light diffusion trough the sample (Figure 3.6a). The software Meta Vue, Meta Imaging Series from Universal Imaging Corporation (CA) was programmed to take pictures (with a Nikon DXM 1200 Digital Camera) every 60.5 micrometers using a 10X magnification lens and dark phase contrast. Numbers of bacteria were counted in five squares (75µm x 75µm) per layer using the Java application ImageJ 1.33u from the National Institute of Health (MD) (Figure 3.6b). Population (P in %) of bacteria per unit of depth (in mm) was calculated in relation of the total number of bacteria counted at each hour. All the microscopy studies were carried out in the Microscopy and Imaging Center of the Biology Department at the Texas A&M University campus.

3.5 Experimental Design

For experimental design of kinetic growth models it is necessary to understand the following concepts: (a) factor, which is an independent variable or design condition as temperature or pH that takes more than one value; (b) treatment, a unique combination of factors and their levels like pH 6.5 and 25ºC; (c) response, known as the dependent variable and is what is measured, e. g. viable count; and (d) parameter, a term in a model that is applied to the value of a factor to obtain the prediction (Legan and Vandeven, 2000). Table 3.2 lists all the different experiments carried out in this research project and the application of these concepts.
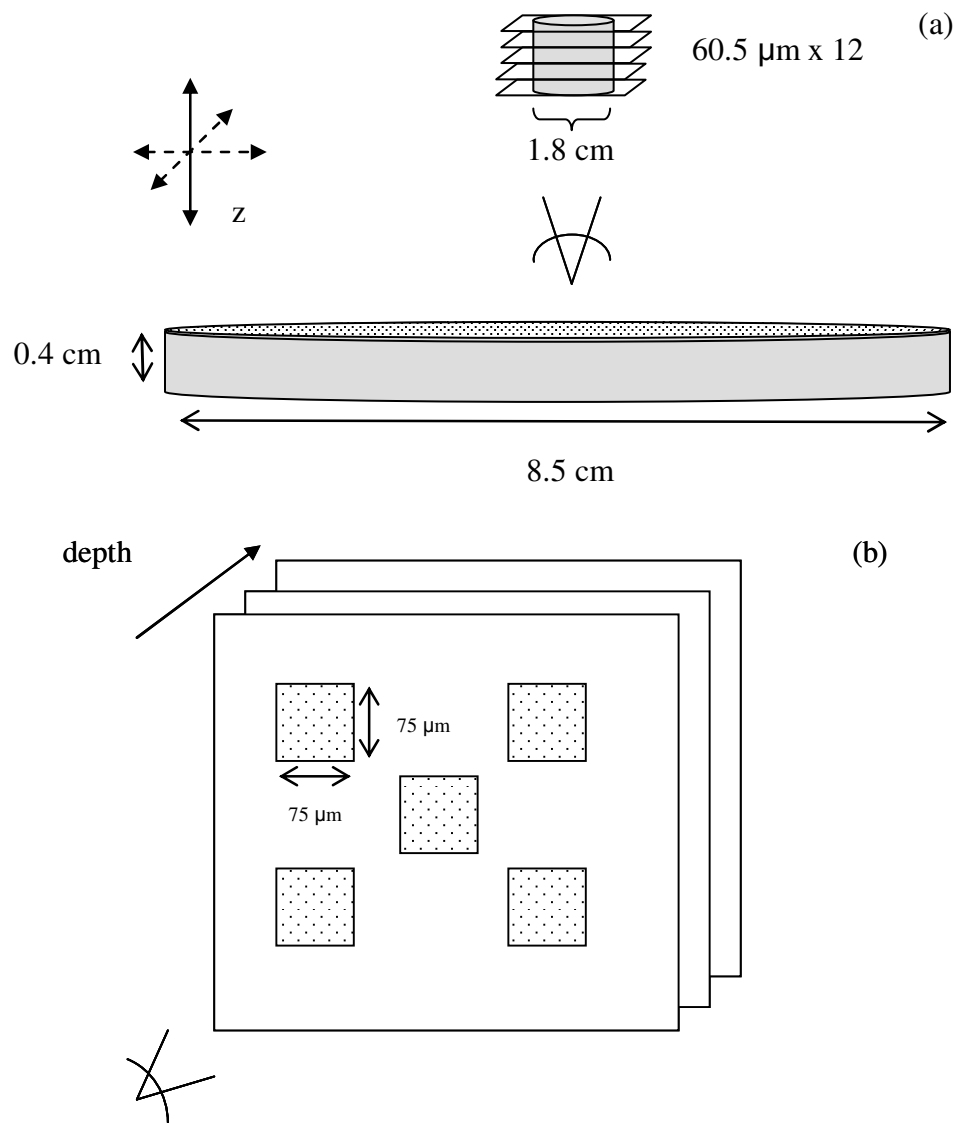
Figure 3.6. Sample design for evaluation of vertical motility (through the height of the gel cylinders) of *E. coli* K-12 MG1655 inoculated on gelatin cylinders using light microscopy. (a) inoculation and extraction of gel cylinder and placement under the microscope, (b) Sample design for counting of bacteria.

Table 3.2

Experimental design

Response	Parameter	Factor	Treatment	R*	Statistical Analysis
Surrogate Selection	D_{10} values (doses: up to 1.0 kGy, increments of 0.2 kGy)	8 bacteria strains	Five doses and one control	3	Linear Regression and Analysis of Covariance (Slope, Coefficient of Variability, CV, and Regression coefficient, R^2)
Harvesting method	Amount of inoculum recovered (%)	Two gel systems and two methods	Melted gel, stomached gel and agar	3	Means Comparison (SNK test)
Penetration depth	Survivors ($\log_{10}$ CFU/ml)	Four slices (*T*, *MT*, *MB*, *B*)	Two systems (irradiated, and non-irradiated)	3	Means Comparison within systems (slices) and between treatments (SNK)
Sample dimension and placement	D_{10} values (kGy)	Five doses (Control and 0.25, 0.5, 1.0, 2.5 and 5.0 kGy)	Five dimensions and placements (*5C1*, *1C1*, *1C5*, *1C25* and *1C25M*)	5	Linear Regression and Analysis of Covariance (Slope, Coefficient of Variability, CV, and Regression coefficient, R^2)
Maintenance at low temperature	Survivors (CFU/ml)	Four days	4 treated and non-treated systems (*G, ER. MR, R*)	5	Comparison within systems and treatments (SNK)

Table 3.2 Continued,

Response	Parameter	Factor	Treatment	R*	Statistical Analysis
Storage temperature	Survivors (CFU/ml)	Four days	Temperatures (4°C, 10°C and 20°C)	5	Comparison within systems (days) and treatments (SNK)
D_{10} values depending on maturity levels	D_{10} values (kGy)	Four gel systems (*C*, *ER*, *MR* and *R*)	Doses (up to 1.2 in increments of 0.3 kGy)	5	Linear Regression and Analysis of Covariance (Slope, Coefficient of Variability, CV, and Regression coefficient, R^2)
Recovery of bacteria at high temperature	Population ($\log_{10}$ CFU/ml)	Four maturity levels	0, 16, 22, 28 and 60 hours after treatment	6	Calculation of *GT* in the region of faster growth and Analysis of Covariance
Surrogate confirmation	D_{10} values (kGy)	Three pathogens	Four doses (up to 1.2 in increments of 0.3 kGy)	5	Linear Regression and Analysis of Covariance (Slope, Coefficient of Variability, CV, and Regression coefficient, R^2)
Validation in real cantaloupes		Most resistant pathogen and surrogate	Four doses (up to 1.2 in increments of 0.3 kGy)	5	Linear Regression and Analysis of Covariance (Slope, Coefficient of Variability, CV, and Regression coefficient, R^2)
Physical properties	Strength, Elasticity, Maximum Strain and Toughness	Four sugar systems and effect of irradiation	Four days	5	Comparison in time within systems (days after treatment) and between treatments (SNK)

Table 3.2 Continued,

Response	Parameter	Factor	Treatment	R*	Statistical Analysis
Physical properties	Strength, Elasticity, Maximum Strain and Toughness	One system (*MR*) and three storage temperatures (4, 10 and 20˚C)	Four days	5	Comparison in time within systems (days after treatment) and between treatments (SNK)
Bacterial mobility (Microbial analysis)	% of initial inoculum	Two gel systems (gelatin and agar)	Four slices, five separated times (every 30min)	3	Comparison in time within layers (hours after treatment) and between treatments (SNK)
Bacterial horizontal mobility (microscopy)	Distance traveled, speed and area of movement. Relationship between speed and area traveled	Time	Three times (every hour) Eigth bacterias at different cardinal positions	8	Comparison in time within calculated parameters (SNK)
		Speed and area traveled		18	Linear Regression and Analysis of Covariance (Slope, Coefficient of Variability, CV, and Regression coefficient, R^2)
Bacterial vertical mobility (microscopy)	% of microbes	Time and Depth (or distance from the center)	Three hours (1 hour interval) 0 h and mixed control, depth 40 μm	5	Comparison of populations within layers at each time (SNK)

* Number of replicates.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preliminary studies

4.1.1 Surrogate Selection

4.1.1.1 Radiation D_{10} *values*

The resulting radiation D_{10} values for the pathogenic and non-pathogenic strains ranged between 1.92 and 0.12 kGy. The pathogenic strains *Salmonella Poona* and *Escherichia coli* O157:H7 933 had significantly (p > 0.0001) different radiation D_{10} values (0.38 and 0.36 kGy, respectively) while *Listeria monocytogenes* ATCC 51414 had a significantly higher radiation D_{10} value (1.09 kGy), making it the most resistant pathogen to irradiation, thus it was selected the target pathogen for this study (Tables 4.1 and 4.2). These results were in agreement with those reported by different researchers (Appendix A), which suggest that *Listeria monocytogenes* should have higher radiation D_{10} values than *E. coli* O157:H7 and *Salmonella Poona*.

From the five non-pathogenic strains evaluated *Enterobacter aerogenes* B199A (D_{10} = 1.92 kGy) was the most resistant non-pathogenic strain, statistically (p > 0.0001) different from the second most resistant strain: *Escherichia coli* K-12 MG1655 (D_{10} = 0.88 kGy) (Table 4.2). The use of *Enterobacter aerogenes* B199A as an indicator of decontamination may be limited, because it will require more energy (higher dose) to be eliminated, which increases the processing cost. This is the principle by which the pasteurization protocols are designed. On the other hand, *Salmonella* LT2 (D_{10} = 0.12

kGy) was the least resistant non-pathogenic strain (Table 4.2), being significantly different (p > 0.0001) from all the strains evaluated.

The radiation D_{10} values of the two *Listeria innocua* strains evaluated, NRRL B-3314 (D_{10} = 0.72 kGy) and NRRL B-3303 (D_{10} = 0.66 kGy), showed not significant differences (p < 0.0001) with the radiation D_{10} values of *Escherichia coli* K-12 MG1655 (D_{10} = 0.88 kGy). These two species are biologically different (gram positive and negative respectively), which suggests that under irradiation treatments there is not a clear difference between gram staining classification (which is based on cell wall structure) under irradiation.

The non-pathogenic strain *Escherichia coli* K-12 MG1655 showed statistically (p > 0.0001) similar resistance to irradiation (D_{10} = 0.88 kGy) when compared with *Listeria monocytogenes* ATCC 51414 (D_{10} = 1.09 kGy). An analysis of covariance demonstrated that there were statistical differences (p > 0.0001) between the linear regressions projected for these strains when compared to *Salmonella* Poona (D_{10} = 0.38 kGy) and *E. coli* O157:H7 933 (D_{10} = 0.36 kGy) (Tables 4.1 and 4.2).

From a different viewpoint, the reduction rate constant (k) from the initial population of the pathogens varied between 6.8 and 1.6 kGy^{-1} (Table 4.1). The rate of reduction in the population of *Listeria monocytogenes* ATCC 51414 (k = 1.6 kGy^{-1}) was less than half than the constant for *Salmonella* Poona (k = 5.0 kGy^{-1}) and *Escherichia coli* O157:H7 933 (k = 6.8 kGy^{-1}) (Table 4.1). The values of the reduction rate constant (k) in the non-pathogenic strains ranged between 0.7 and 25.4 kGy^{-1}, being *Salmonella* LT2 an extreme case (k = 25.4 kGy^{-1}) (Table 4.2).

Table 4.1

Radiation D_{10} values and reaction rate constants (k) for selected pathogenic strains irradiated in gel-based systems

Strain	Decimal reduction (D_{10}) kGy	Linear regression coefficient (R^2)	Coefficient of Variability (CV) %	Reaction rate constant (k) kGy^{-1}
Listeria monocytogenes ATCC 51414	1.09[a]	0.927	3.27	1.6
Salmonella Poona	0.38[b]	0.628	5.07	5.0
Escherichia coli O157:H7 933	0.36[b]	0.822	6.70	6.8

[a-b] Different letters are mean values significantly different (p < 0.0001), n = 3.

R^2, Linear regression coefficient is an indicator of the linear relation between two variables (1.0 means perfect linear regression).

CV, Coefficient of Variability is a measure of the variability of a measured parameter expressed relative to the magnitude of the mean.

Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration using a 2.0 MeV Van De Graaff linear accelerator at 20°C (dose range: 0 to 1.0 kGy, dose rate: 0.5 –1.5 Gy/s).

Table 4.2

Radiation D_{10} values and reaction rate constants (k) for selected non-pathogenic strains irradiated in gel-based systems

Strain	Decimal reduction (D_{10}) kGy	Linear regression coefficient (R^2)	Coefficient of Variability (CV) %	Reaction rate constant (k) kGy^{-1}
Enterobacter aerogenes B199A	1.92[a]	0.860	5.57	0.7
Escherichia coli K-12 MG1655	0.88[bc]	0.904	2.28	2.3
Listeria innocua NRRL B-33314	0.72[c]	0.919	9.22	3.2
Listeria innocua NRRL B-33003	0.66[c]	0.771	4.60	3.8
Salmonella LT2	0.12[d]	0.927	3.27	25.4

[a-b] Different letters are mean values significantly different ($p < 0.0001$), n = 3

R^2, Linear regression coefficient is an indicator of the linear relation between two variables (1.0 means perfect linear regression).

CV, Coefficient of Variability is a measure of the variability of a measured parameter expressed relative to the magnitude of the mean.

Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration using a 2.0 MeV Van De Graaff linear accelerator at 20°C (dose range: 0 to 1.0 kGy, dose rate: 0.5 –1.5 Gy/s).

Several studies (McKellar, 2003; Peri et al, 2002; Kamat and Nair, 1996) suggest that *L. innocua* is a suitable surrogate of *L. monocytogenes*. Peri et al. (2002) determined that *L. innocua* 137 was a good surrogate of *L. monocytogenes* at different pH (3.0, 3.5, 4.0, 9.5, and 10.0) and low water activity (less than 0.92). Kamat and Nair (1996) used a strain of *L. innocua* (F5646 and F5643) to compare the response with four *L. monocytogenes* strains (ATCC 35152, ATCC 35152 ½a, L5458 ½b, and L5562 b2) under heat, gamma radiation, lactic acid, and sodium nitrite treatments. The study determined that, in all the treatments, the survival responses of all the six strains were similar; therefore, they could be used as biological indicators in meat processing treatments.

The current study demonstrated that there may be variations in resistance to ionizing irradiation among *L. innocua* strains, such as *L. innocua* 33314 (D_{10} = 0.72 kGy) and 33003 (D_{10} = 0.66 kGy), which cannot be used as surrogates of *L. monocytogenes* (D_{10} = 1.09 kGy) under electron beam irradiation (Tables 4.1 and 4.2).

In conclusion, *E. coli* K12 MG1655 (D_{10} = 0.88 kGy) is a suitable surrogate of *L. monocytogenes* (D_{10} = 1.09 kGy) for use in studies of electron beam irradiation in the gel-based food model system. In addition, because of its relatively high radiation resistance the surrogate can be used as an indicator of reduction in populations for *Salmonella Poona* (D_{10} = 0.38 kGy) and *E. coli* O157:H7 (D_{10} = 0.36 kGy). This hypothesis was confirmed in experiments that suggest that similar radiation D_{10} values can be obtained in gel systems as in food products with similar composition.

4.1.2 Harvesting method

The recovered population of *E. coli* K-12 MG1655 from the melted gelatin systems (7.2 $\log_{10}$ CFU/ml ± 0.292) was not statistically different (p > 0.005) from stomached gelatins (7.1 $\log_{10}$ CFU/ml ± 0.112) and agar (6.81 $\log_{10}$ CFU/ml ± 0.143). These results constituted 101.5%, 99.1% and 96.1% of the inoculated population, respectively, which suggests that the melting step of the gelatins is a reliable method to harvest bacteria from inoculated gels without the need for special equipment (i. e. stomacher) and the acquisition of additional materials (i. e. stomacher bags or swabs).

4.1.3 Irradiation treatments

4.1.3.1 Penetration depth

A significant reduction (p > 0.001) of 1.8 $\log_{10}$ reductions in CFU/ml, in the inoculated population of *E. coli* K-12 MG1655 was detected only at the top 0.25 cm (*T* slice) of the treated gelatin cylinder at 1.0 kGy, while no significant differences (p < 0.001) were observed between treated and non-treated cylinders at the deeper locations (0.25 cm to 1.0 cm) (Figure 4.1). These results were later confirmed by the calculation of the dose distribution within the gel using Monte Carlo Simulation methods (Kim, 2005), which predicted a uniform dose penetration at the top 0.25 cm (Figure 4.2) when the sample was placed perpendicularly to the beam, at a 67.5° angle from the horizontal position and at 15.25 cm from the radiation source. These results confirmed that modifying the gelatin dimensions (i. e., decreasing and diameter) was the solution to achieve a higher reduction rate or population numbers (*k* or *Ki* at a particular dose). In
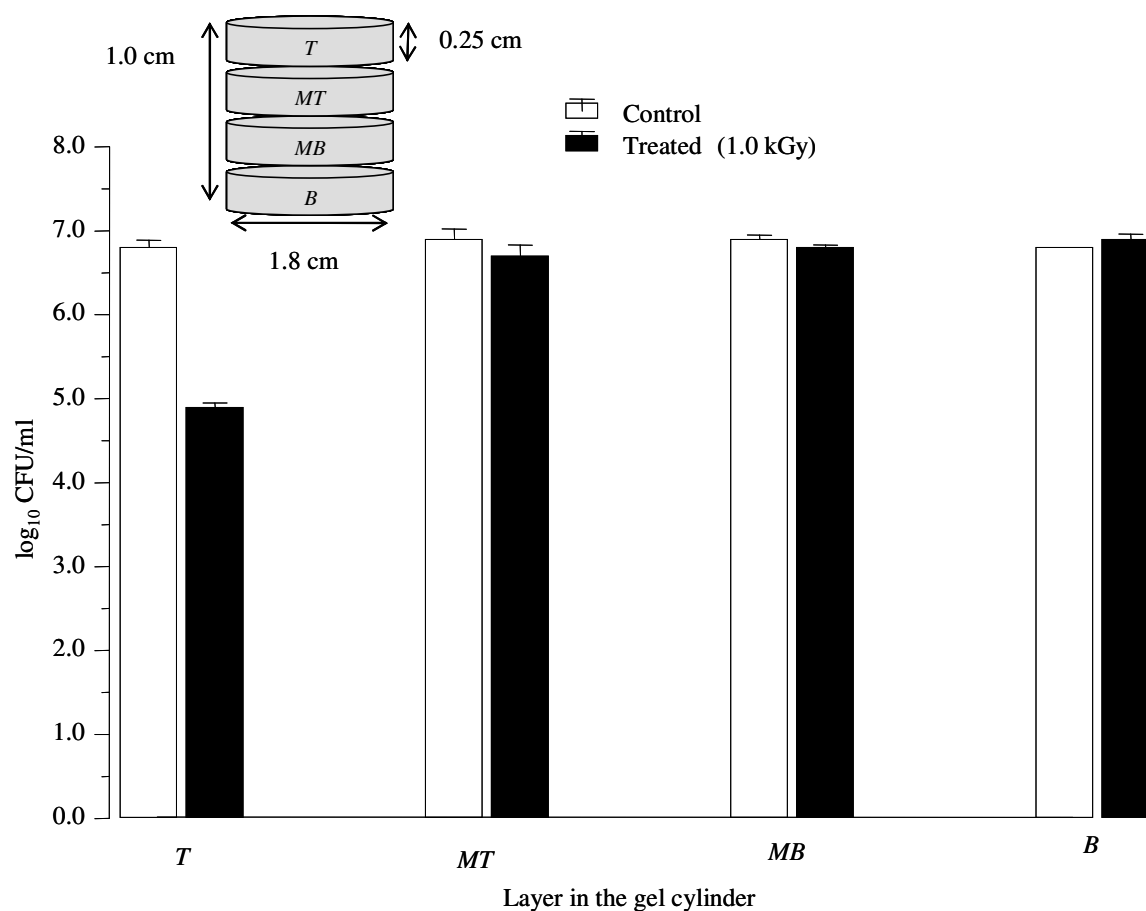
Figure 4.1. Distribution of *Escherichia coli* K-12 MG1655 on irradiated (1.0 kGy) and non-irradiated (Control) gelatin systems.

Positions refer to a 0.25 cm distance from the top surface (*T*) to the bottom (*B*) of the 1.8 cm diameter cylinders. Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration using a 2.0 MeV Van De Graaff linear accelerator at 20°C (dose range: 0 to 1.0 kGy, dose rate: 0.5 –1.5 Gy/s).
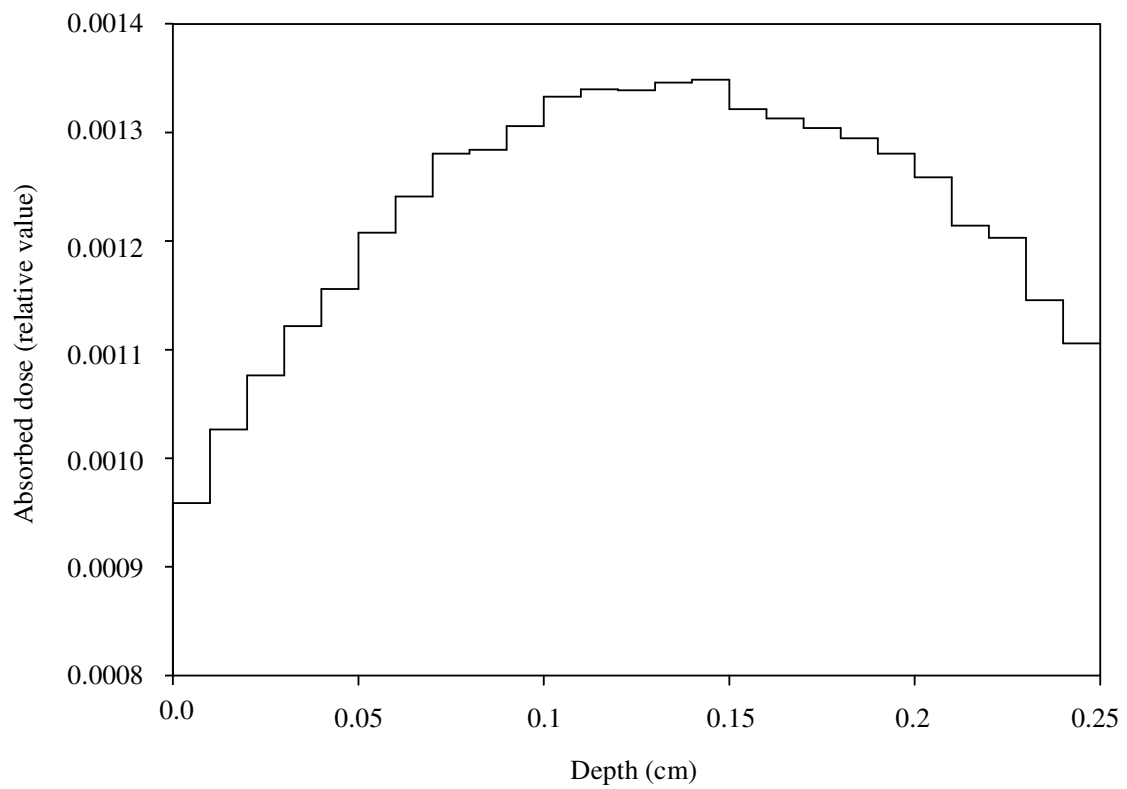
Figure 4.2. Dose distribution within the gel cylinders predicted using Monte Carlo simulation techniques. (Adapted from Kim, 2005).

other words a decrease in the radiation D_{10} values (dose required to eliminate 1.0 $\log_{10}$ CFU/ml) of the inoculated microbes when using a 2.0 MeV Linear Accelerator.

4.1.3.2 Sample configuration and placement

No differences on microbial reduction characteristics (radiation D_{10} values) occurred when the height of the gelatin cylinder was reduced from 1.0 cm (*1C1*) to 0.5 cm (*1C5*), while the radiation D_{10} value decreased by a factor of 3 when the height was further reduced from 1.0 cm to 0.25 cm (*1C25*) (Table 4.3). When the diameter of the cylinder was reduced from 8.5 to 6.0 cm (Figure 3.3), for the *1C25M* configuration, the microbial reduction had almost a six-fold decrease, and no microbes were detected at doses of 2.5 and 5.0 kGy. This result suggests that commercial sterilization was achieved (no microbial counts), but complete sterilization cannot be guaranteed. Therefore, the linear regression for this configuration (*1C25M*) was determined from the survivors on gel systems treated at doses less than 1.2 kGy (Table 4.3 and Figure 4.8). The radiation D_{10} values obtained in the modified configuration were in agreement with those reported in literature for other food systems (Appendix A).

4.1.4 Recovery and maintenance

4.1.4.1 Maintenance at low temperatures

The composition of the gelatin-based model food systems had no significant effect on the reduction of the initial population of *E. coli* K-12 MG1655 when stored at 4°C. Reductions of 1.2 ± 0.15, 1.4 ± 0.08 and 1.3 ± 0.08 $\log_{10}$ (CFU/ml) were achieved

Table 4.3

Radiation D_{10} values and reaction rate constants (k) for *Escherichia coli* K-12 MG1655 under electron beam irradiation as a function of sample positions and dimensions using a 2.0 MeV Van De Graaff linear accelerator

Sample	Configuration	Decimal reduction (D_{10}) kGy	Linear regression coefficient (R^2)	Coefficient of Variability (CV) %	Reaction rate constant (k) kGy^{-1}
5C1	5 cylinders – 1.0 cm height x 1.8 cm diameter, 22.5°, 30.5 cm from beam exit	0.88[c]	0.904	2.28	2.3
1C1	1 cylinder – 1.0 cm height x 8.0 cm diameter, 22.5°, 30.5 cm from beam exit	3.01[a]	0.872	4.196	0.8
1C5	1 cylinder – 0.5 cm height x 8.0 cm diameter, 22.5°, 30.5 cm from beam exit	2.84[a]	0.862	4.147	0.8
1C25	1 cylinder – 0.25 cm height x 8.0 cm diameter, 22.5°, 30.5 cm from beam exit	1.19[b]	0.976	4.374	1.9
1C25M	1 cylinder – 0.25 cm height x 6.0 cm diameter, 67.5°, 15.25 cm from beam exit	0.21[d]	0.986	7.510	11.4

[a-c] Different letters are mean values significantly different ($p < 0.0001$), n = 5.

R^2, Linear regression coefficient is an indicator of the linear relation between two variables (1.0 means perfect linear regression).

CV, Coefficient of Variability is a measure of the variability of a measured parameter expressed relative to the magnitude of the mean.

at 1.0 kGy in *ER*, *MR* and *R* systems, respectively (Figures 4.3 to 4.5). Therefore the presence of sugars did not affect the microbial damage induced by irradiation.

The number of *E. coli* K-12 MG1655 survivors ($\log_{10}$ CFU/ml) in non-irradiated systems (*C*) remained practically constant throughout the period of study (3 days) (Figure 4.3). The two *ER* systems showed a significant ($p > 0.005$) decrease by day 1 (1.0 ± 0.16 $\log_{10}$ CFU/ml) and day 2 (0.5 ± 0.21 $\log_{10}$ CFU/ml), which suggests that the nutrients present in these systems (fructose and glucose) could act as inhibitors of the maintenance or growth at low temperatures (4°C) regardless of irradiation treatments. The populations (CFU/ml) in *MR* systems remained constant in numbers, with no significant ($p > 0.005$) changes during a four-day period, and the *R* systems showed a significant ($p > 0.005$) increase (0.6 ± 0.25 $\log_{10}$ CFU/ml) at day 3 (Figures 4.4 and 4.5). The *R* and *MR* sugar systems differ from *ER* systems by the presence of fructose (Table 3.1) in which no increase in populations was observed (Figure 4.3 and Table 4.4). This suggests that at low temperature (4°C) the increase in sucrose may promote the growth of bacteria in non-irradiated samples (*R*).

From these results we can conclude that storage of irradiated samples at 4°C could help prevent recovery or growth of *E. coli* K-12 MG1655 regardless of their sugar content.

4.1.4.2 Storage at different temperatures

As expected, the irradiated microbial populations (1.0 kGy) of *E. coli* K-12 MG1655 were able to recover at higher storage temperatures. No significant changes were observed in moderately ripe Irradiated (*MRI*) systems stored at 10°C (Figure 4.6).
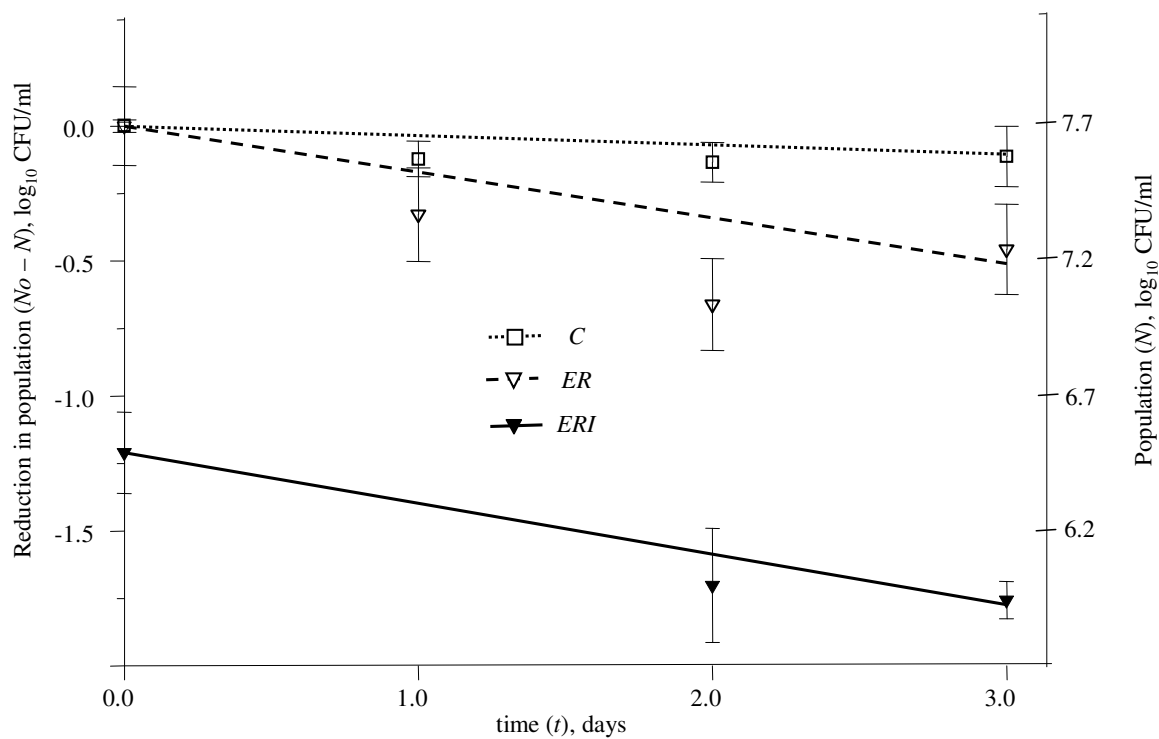
Figure 4.3. Survival of *Escherichia coli* K-12 MG1655 in non-irradiated *(ER)* and irradiated (*ERI*) early ripe (3 % w/v, 1.5 glucose: 1.5 fructose : 0 sucrose) gel-based systems stored at low temperature (4ºC).

Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration. Irradiation dose: 1.0 kGy, at room temperature (20ºC) using a 2.0 MeV Van De Graaff linear accelerator (n = 5).

Figure 4.4. Survival of *Escherichia coli* K-12 MG1655 in non-irradiated *(MR)* and irradiated (*MRI*) at 1.0 kGy moderately ripe (5.5 % w/v, 1 glucose: 1 fructose : 1 sucrose) gel-based systems stored at low temperature (4ºC).

Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration. Irradiation dose: 1.0 kGy, at room temperature (20ºC) using a 2.0 MeV Van De Graaff linear accelerator (n = 5).

Figure 4.5. Survival of *Escherichia coli* K-12 MG1655 in non-irradiated *(R)* and irradiated *(RI)* at 1.0 kGy ripe (8 % w/v, 0.5 glucose: 0.5 fructose : 2 sucrose) gel-based systems stored at low temperature (4ºC).

Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration. Irradiation dose = 1.0 kGy, at room temperature (20ºC) using a 2.0 MeV Van De Graaff linear accelerator (n = 5).

However, a sharp increase (1.0 $\log_{10}$ CFU/ml) of the initial population (*A*) occurred after 16 hours (*L*) at 20°C (estimated *GT* for irradiated populations of 2.3 h and 1.5 h for the control) and equilibrium was reached after 48 hours (Figure 4.7) at that temperature. These results suggest that the higher storage temperature (20°C) provided conditions enough for the bacteria to recover back to their initial population numbers in a shorter period of time (Table 4.4). In addition, the increase in the bacteria numbers in the *MR* gel system suggests that the populations in the *1C5* configuration (amount of gel, sample dimension and inoculum size) were able to recover their initial numbers (*A*) and even 1.0 $\log_{10}$ CFU/ml higher (Figure 4.7).

4.2 Radiation D_{10} values using optimum configuration

Because the population in the systems subjected to the highest doses (2.5 and 5.0 kGy) could not be measured, an analysis of the means revealed that when the gel-based systems were irradiated at 0.5 kGy in the optimum configuration (*1C25M*) the population reduction was significantly ($p < 0.0001$) higher in the *MR* systems (2.3 ± 0.17 $\log_{10}$ CFU/ml). No statistical differences ($p > 0.0001$) were found among *C*, *ER* and *R* systems (1.4 ± 0.16, 1.3 ± 0.08 and 1.5 ± 0.20 $\log_{10}$ CFU/ml respectively). At 1.0 kGy the reduction (*Ki*) in population in early ripe (*ER*) systems (2.5 ± 0.13 $\log_{10}$ CFU/ml) was significantly ($p > 0.0001$) higher compared with the system without sugars (*C*): 1.9 ± 0.05 $\log_{10}$ CFU/ml, and those with high sugar content (*R*): 2.0 ± 0.04 $\log_{10}$ CFU/ml.

When the food systems were irradiated up to 5.0 kGy using the optimum configuration (*1C25M*) doses higher than 2.5 kGy killed the inoculated population (6.0 to 7.0 $\log_{10}$ CFU/ml), which led us to conclude that this dose level is sufficient to
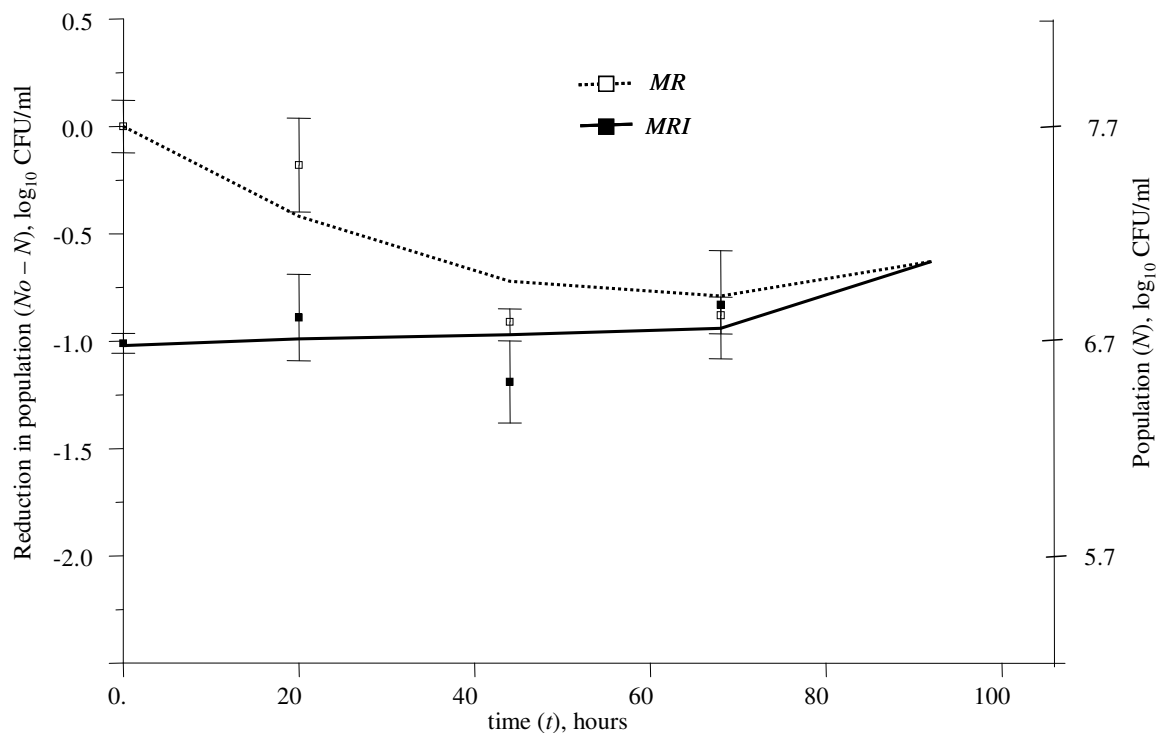
Figure 4.6. Survival of non-irradiated (*MR*) and irradiated (*MRI*) *Escherichia coli* K-12 MG1655 in moderately ripe (5.5 % w/v, 1 glucose: 1 fructose : 1 sucrose) gel-based systems stored at 10ºC.

Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration. Irradiation dose: 1.0 kGy, at room temperature (20ºC) using a 2.0 MeV Van De Graaff linear accelerator (n = 5).
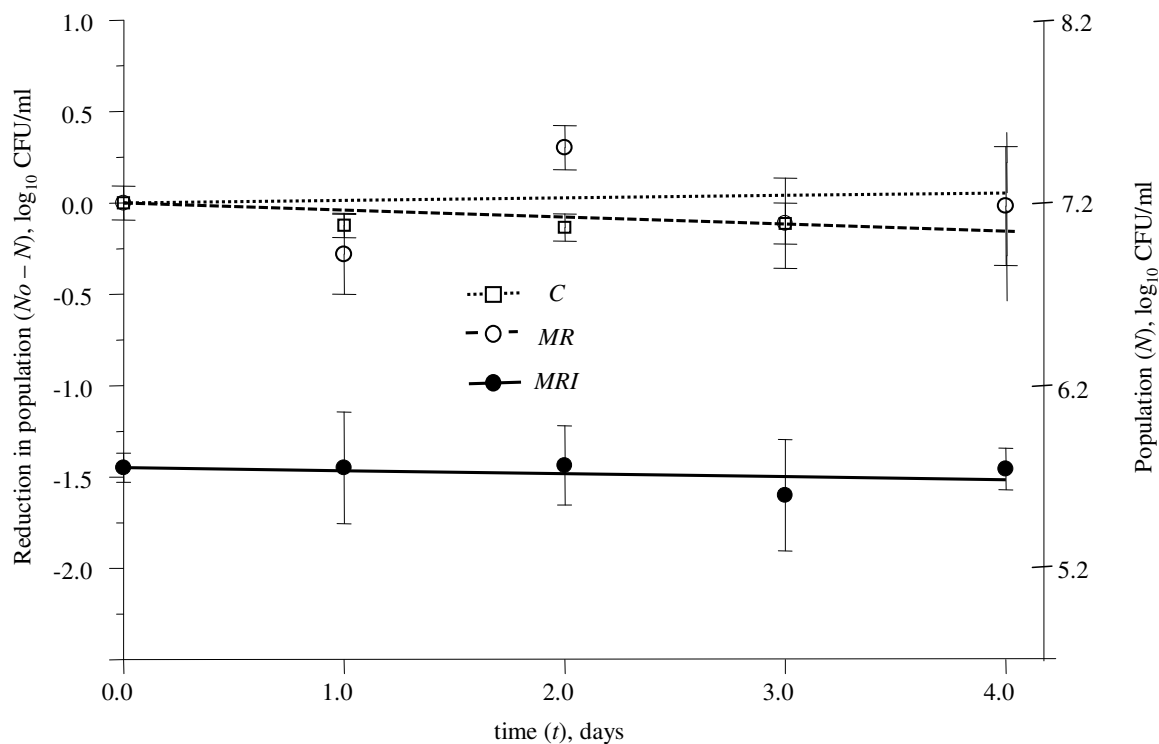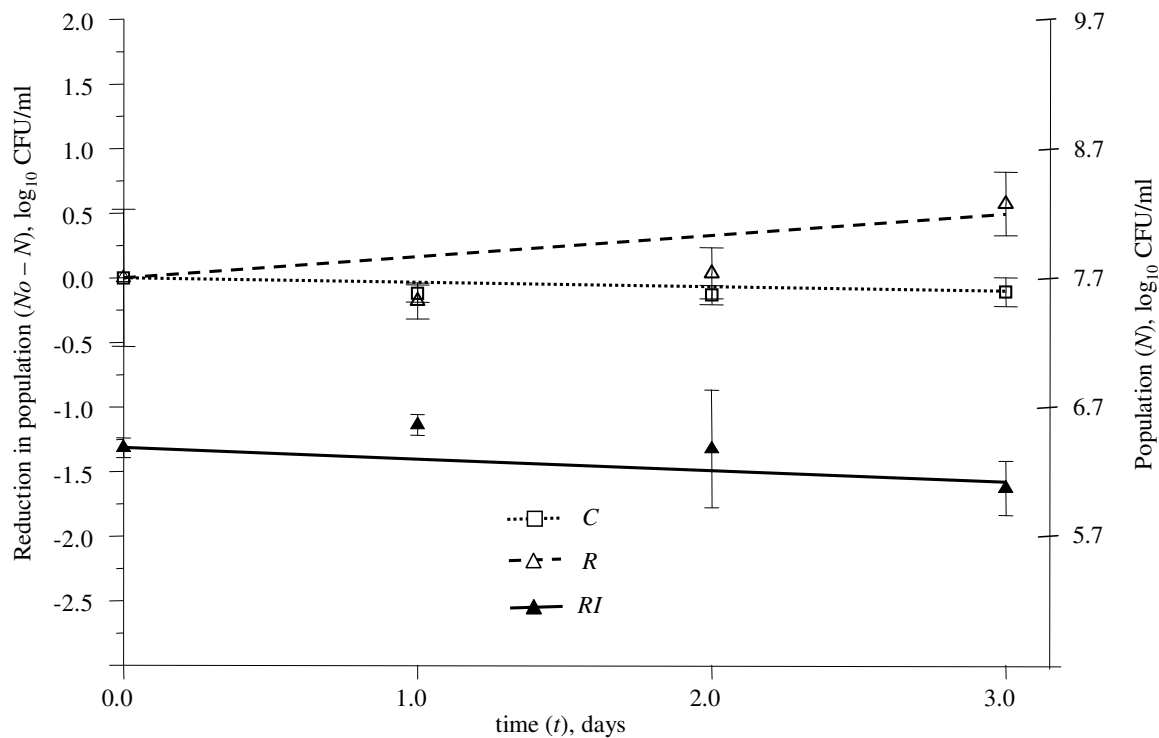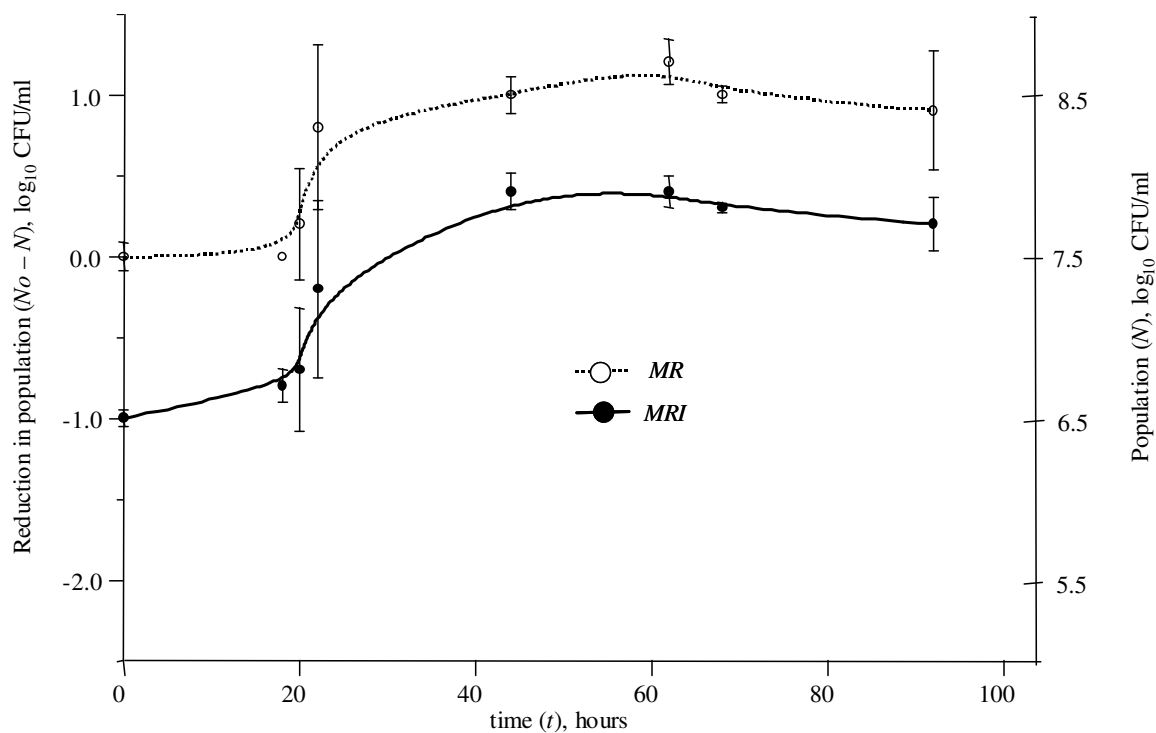
Figure 4.7. Survival of non-irradiated (*MR*) and irradiated (*MRI*) *Escherichia coli* K-12 MG1655 in moderately ripe (5.5 % w/v, 1 glucose: 1 fructose : 1 sucrose) gel-based systems stored at 20ºC.

Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration. Irradiation dose: 1.0 kGy, at room temperature (20ºC) using a 2.0 MeV Van De Graaff linear accelerator (n = 5).

Table 4.4

Maintenance and recovery of populations of *Escherichia coli* K-12 MG1655 in non-irradiated and irradiated (1.0 kGy) gel systems stored at different temperatures for three days

Treatment	Storage Temperature (°C)	Change in the microbial population, $\log_{10}$ CFU/ml per day*	Linear regression coefficient (R^2)
Control - no sugars *(C)*	4	-0.03	0.529
Early Ripe *(ER)*	4	-0.17	0.628
Early Ripe Irradiated *(ERI)*	4	-0.19	0.943
Moderately Ripe *(MR)*	4	0.01	0.010
Moderately Ripe Irradiated *(MRI)*	4	-0.02	0.142
Ripe *(R)*	4	0.16	0.625
Ripe Irradiated *(RI)*	4	-0.09	0.598
Moderately Ripe *(MR)*	10	-0.35	0.856
Moderately Ripe Irradiated *(MRI)*	10	0.03	0.046
Moderately Ripe *(MR)*	20	*GT* = 1.5 h	0.999
Moderately Ripe Irradiated *(MRI)*	20	*GT* = 2.3 h	0.979

GT: Generation time, hours, Eq. (2.12), Eq. (2.17) and Eq. (2.18), Figure 2.1.

R^2: Linear regression coefficient is an indicator of the linear relation between two variables (1.0 means perfect linear regression).

*Microbial counts done using Standard Plate Count techniques (replications: 5).

Samples were irradiated using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator at 20°C.

eliminate microbes (commercial sterilization) in this configuration (Figure 4.8). A comparison of the calculated radiation D_{10} values for the three different sugar systems (*ER*, *MR* and *R*) with those systems without sugars (*C*) revealed that an effect of the sugar content was not significantly (p > 0.001) established using this range (up to 8 %) and ratio of sugars (Table 3.1), and under radiation doses below 1.2 kGy. The radiation D_{10} values in *R* (0.26 kGy), *ER* (0.25 kGy) and *MR* (0.22 kGy) systems were statistically similar (p > 0.001) to those of the *C* systems (0.21 kGy) (Figure 4.8).

From another viewpoint, the reduction rate constant, which is the rate of decay in a population depending on time (in this case dose), can be explained with the *k* values. The values of 11.4, 7.0, 13.3 and 7.4 kGy^{-1} were achieved for *C*, *ER*, *MR* and *R* treatments respectively, which suggests that a higher rate of reduction in the population can be achieved in the *ER* and *R* samples.

If the number of bacteria killed (*Ki*) at 1.0 kGy is analyzed 4.0, 4.0, 3.5 and 4.8 log_{10} CFU/ml can be achieved at 1.0 kGy for *C*, *ER*, *MR* and *R* treatments respectively. Most of the technologies are developed to achieve more than 5.0 log_{10}, which is the difference between the populations encountered regularly in food products (7.0 log_{10} CFU/ml) and the infective dose (1.0 log_{10} CFU/ml). For example, when heat is applied to milk at 71.6 ˚C for 15 seconds, a 5.0 to 6.0 log_{10} CFU/ml of non-spore forming bacterial pathogens occurs, and the resulting product is considered pasteurized (Yousef and Courtney, 2003).

As cited in Section 2.3.5.1 and listed in Appendix A, the expected radiation D_{10} values for the species under analysis in this study should range between 0.10 and
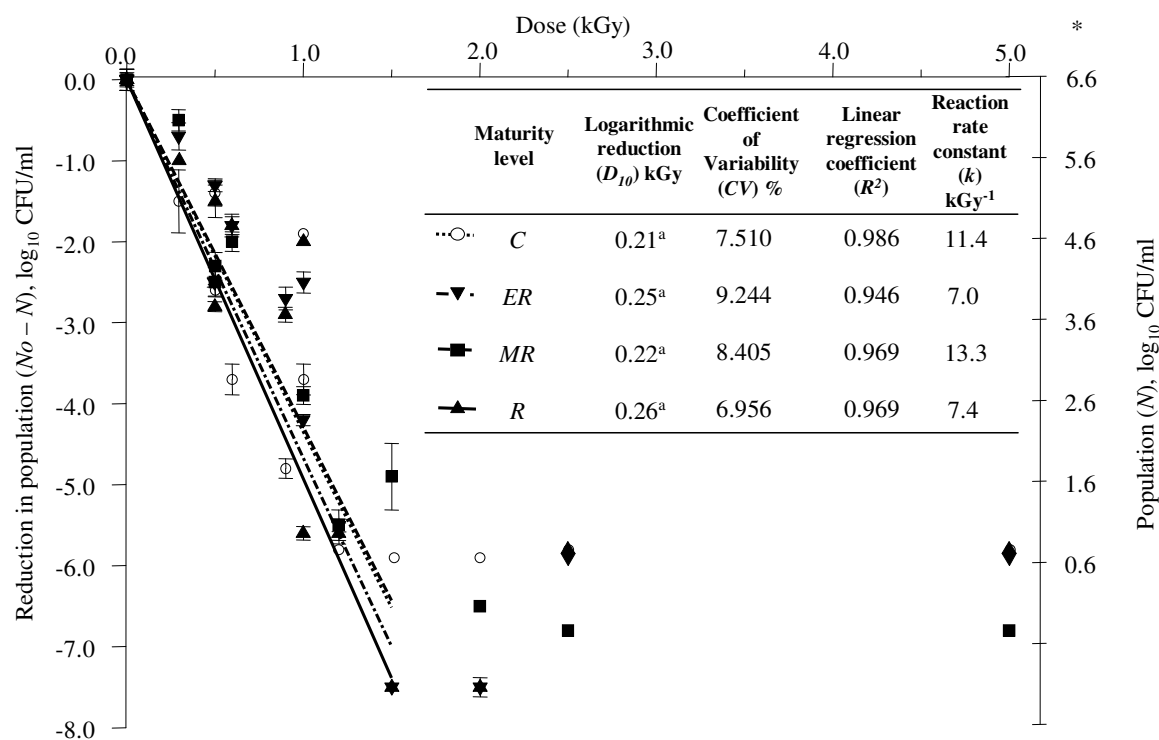
Figure 4.8 Radiation D_{10} values and reduction rate constants (k) for *Escherichia coli* K-12 MG1655 on gel systems depending on sugar content.

[a-b] Same letters are mean values statistically similar (p > 0.0001). Samples were irradiated using the *1C25M* configuration (1 cylinder – 0.25 cm height and 6.0 cm diameter, 67.5°, 15.25 cm from beam exit) under a 2.0 MeV Van De Graaff linear accelerator at 20°C. 5 replications were made per cylinder. *C*: no-sugars, *ER*: 3 %, 1.5:1.5:0, *MR*: 5.5 %, 1:1:1, *R*: 8 %, 0.5:0.5:2 (% total sugars w/v, glucose: fructose : sucrose). R^2, Linear regression coefficient is an indicator of the linear relation between two variables. *CV*, Coefficient of Variability is a measure of the variability of a measured parameter expressed relative to the magnitude of the mean. * Estimated values from different trials.

0.70 kGy even when using highly resistant strains and stressed conditions. The resulting radiation D_{10} values using the modified design (*1C25M*) were similar to those reported by most of the researchers in food irradiation under different conditions. The study that most resemble the conditions used in this experiment is the one made by Stahl et al (2000), who used a 2.2 MeV Van de Graff Electron accelerator (150μA from Vivirad High Voltage Handschuheim, France). The authors reported radiation D_{10} values of 0.49 ± 0.05 kGy and 0.41 ± 0.03 kGy in soft and red smear cheese for *Listeria monocytogenes*. They proved that 2.0 kGy were necessary to eliminate 10^4 bacteria per gram (4.0 $\log_{10}$) of cheese in the surface (3 to 5 mm), and packaging and preservation at 4°C prevented any possible post-processing contamination up to 30 days without sensory changes.

With the exception that the tested system was cheese and the bacteria are different strains, similar results were obtained by treating the surface of a real food system using the same irradiation conditions and penetration depth. The preservation of population reduction under low temperature conditions was also confirmed.

4.3 Recovery of microbes using the optimum configuration

A significantly higher reduction in the microbial population ($p < 0.001$) was achieved in the early ripe systems (*ERI*) treated at 1.0 kGy (4.2 ± 0.1 $\log_{10}$ CFU/ml) compared with the other three systems (3.7 ± 0.2, 3.9 ± 0.1 and 3.7 ± 0.0 $\log_{10}$ CFU/ml for *CI*, *MRI* and *RI* respectively) (Figures 4.9 to 4.12 and Table 4.5). The *ER* systems were the only systems containing reducing sugars (glucose and fructose), which

researchers previously stated to have an antimicrobial effects after irradiation when irradiated as solutions in water (Namiki, 1976).

These results suggest that the antimicrobial effect of sugars may disappear when these sugars are combined with gelatin or sucrose is added to the medium. The anti-microbial effect was not noticed under the irradiation conditions using in this study. A more in depth study (using water as medium and pure sugar concentrations) may reveal more information.

The surviving population (*Su*) grew at a slower rate in the *CI* systems (*GT* = 2.6 h) compared to the *RI* systems (*GT* = 1.6 h) and even at a faster rate in the *ERI* (*GT* = 1.3 h) and *MR* (*GT* = 1.5 h) systems at 20°C (Figures 4.9 to 4.12). The hypothesis that increasing the amount of a nutrient (sugars) increases the growth rate of microbial populations was not confirmed, neither Eq. (2.20) could be used to explain the behavior.

Linear regression analysis revealed that within this range of sugar concentrations (3.0 to 8.0 %) a small increase (3.6 minutes per % of sugar) in the Generation Times (*GT* = 0.062*[S]* + 1.0953, R^2 = 0.991) was noticed when increasing the sugar content (R^2 = 0.991), which means that the population grew slowly at high sugar concentrations. The result implies that sugars may not play a role in the elimination of microbes when using irradiation, but they provide enough nutrients for the microbes to recover from the reduced (*Ki*) populations.

This study demonstrated that the presence of gelatin or sugars was enough for the remaining bacteria (3.0 to 4.0 $\log_{10}$ CFU/ml) to recover to their initial numbers within 60 hours at 20°C. A final population higher than the inoculum (*A*) was measured in the *MRI*

systems (C = 1.0 ± 0.06 $\log_{10}$ CFU/ml) after 60 hours compared with *CI* and *RI* (C = 0.3 ± 0.08 and 0.1 ± 0.02 $\log_{10}$ CFU/ml respectively) (Figures 4.9 to 4.12). In *ERI* the population after 60 hours (C = -0.6 ± 0.08 $\log_{10}$ CFU/ml) did not reach the inoculum numbers. This result suggests that the remaining population was healthy and in good conditions for recovery (*Su*) so the term "repair" does not apply.

The term recovery factor (*RF*), Eq. (2.21), can be applied making the assumption that the irradiated early ripe treatment (*ERI*) was a restricted media and the irradiated moderately ripe (*MRI*) treatment was the optimum media for growth. Thus, the recovery factor (*RF*) after 60 hours was 1.4 (Table 4.5). This means that a 1.4 greater population of bacteria (*C*) can grow in the *MRI* compared to the *ERI*.

Another useful parameter that can be calculated is the number of injured bacteria (*I*). Making the same assumption that *ERI* treatments were a restricted environment and *MRI* the optimum medium, the number of injured populations of bacteria (*I*), or bacteria with the capability to recover only in optimum conditions was 0.3 $\log_{10}$ CFU/ml. This means that there were almost 0.5 $\log_{10}$ (almost the infective dose) of a population of bacteria that can be multiplied if the optimal conditions are provided.

Even when the same inoculum (*A*) was used, the population inoculated in non-irradiated *C* systems decreased (-1.5 ± 0.04 $\log_{10}$ CFU /ml) significantly (p < 0.001) at room temperature (20°C) after 16 hours (Figure 4.9) and *MR* systems (-1.2 ± 0.17 $\log_{10}$ CFU/ml) after 46 hours (Figure 4.11). This may be probably due to the absence of sugars in this system and the growth was smaller compared with the growth observed in the irradiated systems, which suggests that the capacity of the systems (*A*) is about
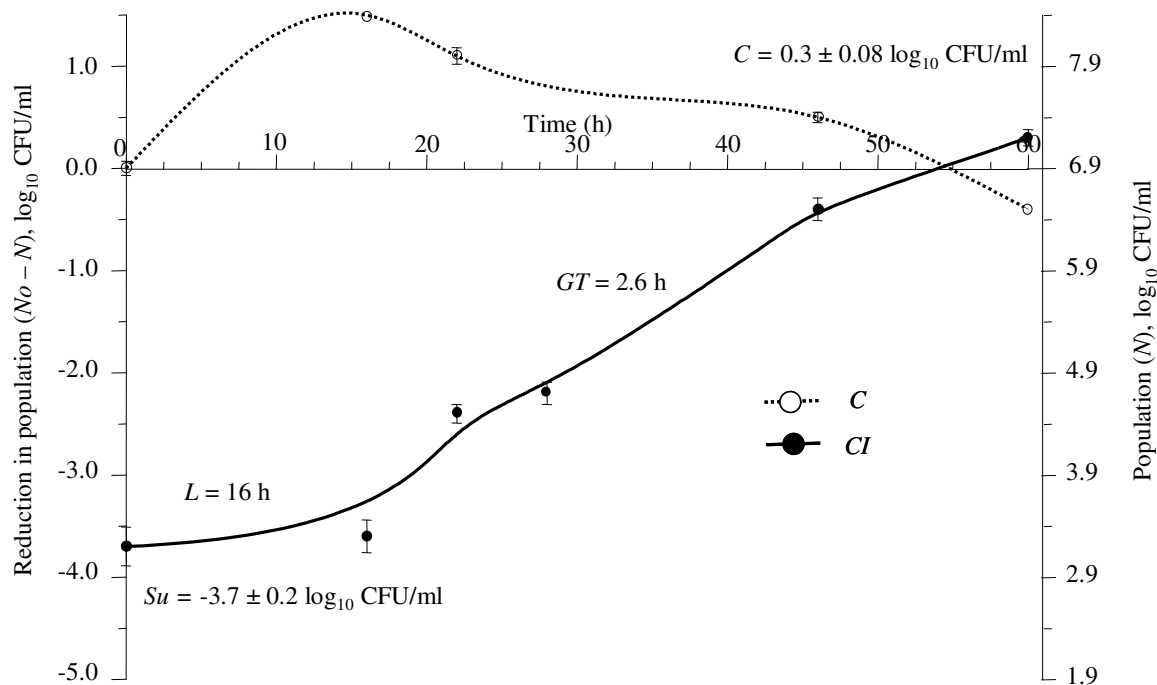
Figure 4.9. Recovery and maintenance of populations of *Escherichia coli* K-12 MG1655 in non-irradiated (*C*) and irradiated at 1.0 kGy (*CI*) gel systems without sugars at room temperature (20°C).

Samples were irradiated using the *1C25M* configuration (1 cylinder – 0.25 cm height and 6.0 cm diameter, 67.5°, 15.25 cm from beam exit) under a 2.0 MeV Van De Graaff linear accelerator at 20ºC. (n = 5).
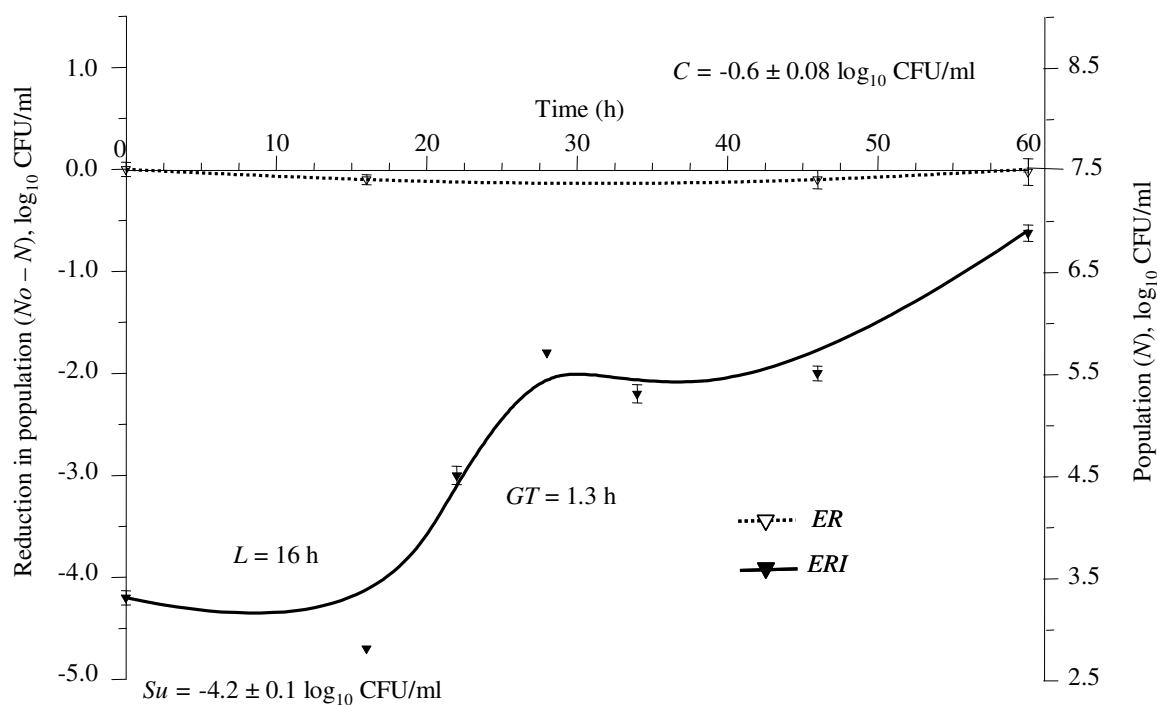
Figure 4.10. Recovery and maintenance of populations of *Escherichia coli* K-12 MG1655 in non-irradiated (*ER*) and irradiated at 1.0 kGy (*ERI*) early ripe (3% w/v, 1.5 glucose: 1.5 fructose : 0 sucrose) gel systems at room temperature (20°C).

Samples were irradiated using the 1C25M configuration (1 cylinder – 0.25 cm height and 6.0 cm diameter, 67.5°, 15.25 cm from beam exit) under a 2.0 MeV Van De Graaff linear accelerator at 20 ℃. (n = 5).
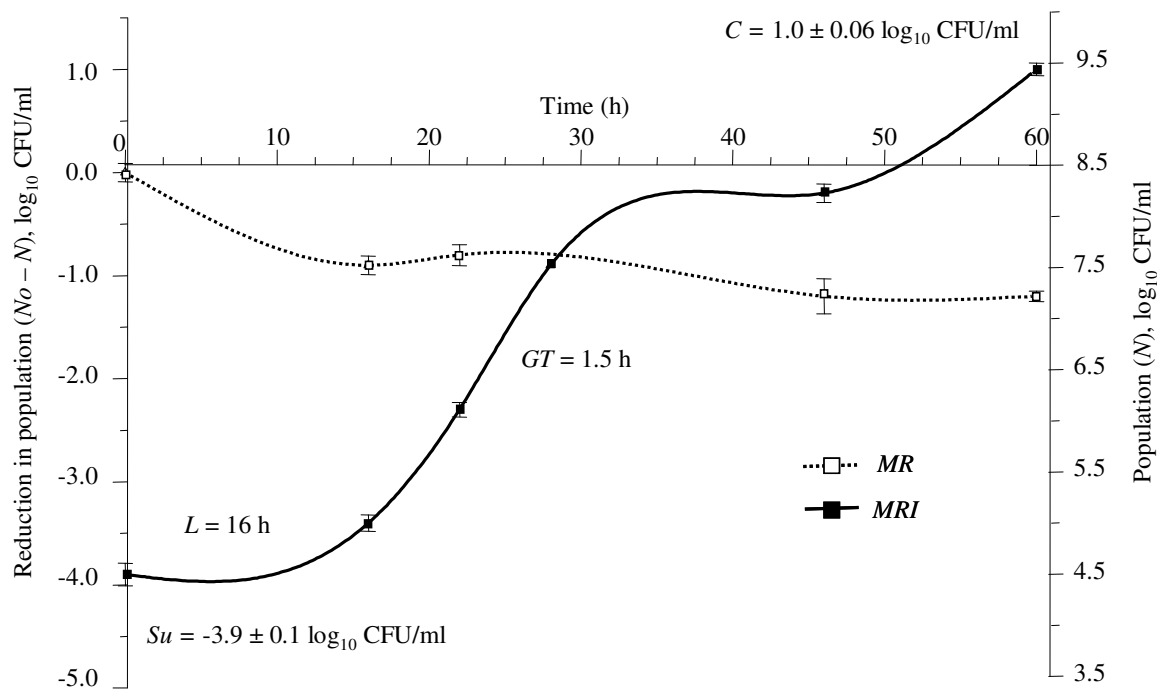
Figure 4.11. Recovery and maintenance of populations of *Escherichia coli* K-12 MG1655 in non-irradiated (*MR*) and irradiated at 1.0 kGy (*MRI*) moderately ripe (5.5% w/v, 1 glucose: 1 fructose : 1 sucrose) gel systems at room temperature (20°C).

Samples were irradiated using the *1C25M* configuration (1 cylinder – 0.25 cm height and 6.0 cm diameter, 67.5°, 15.25 cm from beam exit) under a 2.0 MeV Van De Graaff linear accelerator at 20ºC. (n = 5).
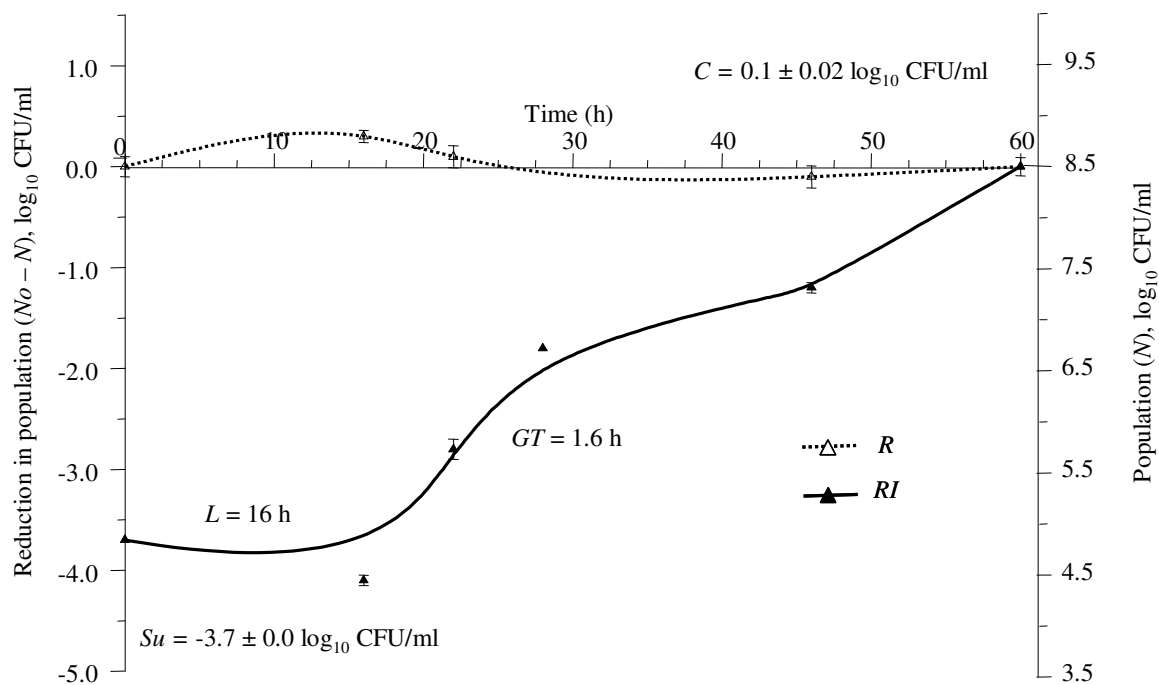
Figure 4.12. Recovery and maintenance of populations of *Escherichia coli* K-12
MG1655 in non-irradiated (*R*) and irradiated at 1.0 kGy (*RI*) ripe (8% w/v, 0.5 glucose:
0.5 fructose : 2 sucrose) gel systems at room temperature (20°C).

Samples were irradiated using the 1C25M configuration (1 cylinder – 0.25 cm height and 6.0 cm diameter,
67.5°, 15.25 cm from beam exit) under a 2.0 MeV Van De Graaff linear accelerator at 20ºC. (n = 5).

Table 4.5

Recovery and maintenance at room temperature (20°C) for 60 hours of irradiated (1.0 kGy) populations of *Escherichia coli* K-12 MG1655 in gel-based systems that resemble fruit maturity levels

Treatment	Sugars (%, Fructose: Glucose: Sucrose)	Reduction in population at 1.0 kGy (*Ki*) $\log_{10}$ CFU/ml	Lag Phase (*L*) h	Generation Times (*GT*) h	Final Population (*C*) $\log_{10}$ CFU/ml
No Sugars (*C*)	Control	$-3.7^a \pm 0.2$	16	2.6^c	$0.3^b \pm 0.08$
Early Ripe (*ER*)	(3.0, 1.5: 1.5:0)	$-4.2^b \pm 0.1$	16	1.3^a	$-0.6^d \pm 0.08$
Moderately Ripe (*MR*)	(5.5, 1:1:1)	$-3.9^a \pm 0.1$	16	1.5^a	$1.0^a \pm 0.06$
Ripe (*R*)	(8.0, 0.5:0.5:2)	$-3.7^a \pm 0.0$	16	1.6^b	$0.1^c \pm 0.02$

[a-b] Different letters are mean values significantly different ($p < 0.0001$), n = 5

Samples were irradiated using the *1C25M* (1 cylinder – 0.25 cm height and 6.0 cm diameter, 67.5°, 15.25 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C).

1×10^8 CFU/ml as most of the food systems and the inoculated population of bacteria was ending its stationary phase.

The growth of *E. coli* species under different conditions has been reported to be similar to the results obtained in this study. A *GT* of 1.1 h in Tryptic Soy Broth (*E. coli* 5 O157:H7), 0.78 h in Ground Mutton (*E. coli* Wild species), 1.3 to 1.8 h on skim milk (*E. coli* 2 EIEC) and 0.63 h on cheese curd (*E. coli* 6 EP) were reported by ICMSF (1996) at similar temperature and pH conditions. This observation suggests that the recovery of this strain in this study was similar to those obtained in other *E. coli* species under optimum conditions.

For the selected surrogate (*E. coli* K-12 MG1655), workers in the *E. coli* genome Project at the University of Wisconsin reported doubling times (Generation Times), Eq. (2.18), of 1.06 h in 0.1% glucose at 37°C and shaking at 225 rpm (EGP, 2004). The authors also reported 1.24 h (0.4% Glucose), 1.07 h (0.4% Glucose), 1.13 h (0.2% Glucose using ATCC 47076), 1.09 h (0.2% Glucose) and 1.06 (0.2% Glucose). All these results were measured using the Optical Density (OD) method at 600 nm of wavelength.

4.4 Validation studies

4.4.1 Irradiation treatments

4.4.1.1 Surrogate confirmation in the modified configuration

Compared to the radiation D_{10} values obtained using other configurations (*5C1*, *1C1*, *1C5*, *1C25*) (Figure 3.3), the three pathogens and the surrogate had significantly lower radiation D_{10} values (three to five times smaller) when subjected to irradiation

using the *1C25M* configuration (Figure 4.13). *Listeria monocytogenes* ATCC 51414 showed similar reduction rate constant ($k = 13.0$ kGy^{-1}) as the selected surrogate: *Escherichia coli* K-12 MG1655 ($k = 11.4$ kGy^{-1}). The population of *E. coli* O157:H7 933 can be significantly reduced at a rate of 15.6 kGy^{-1}, similar to *Salmonella* Poona ($k = 15.9$ kGy^{-1}) (Figure 4.13). These results confirm that *E. coli* K-12 MG1655 is a suitable surrogate for the pathogens of importance in food systems and the calculated radiation D_{10} values are similar to those reported in the literature (Appendix A).

4.4.1.2 Comparison of the model system with real cantaloupes

Escherichia coli K-12 MG1655 had a significantly ($p < 0.0001$) higher radiation D_{10} value (0.45 kGy) when inoculated in cantaloupes than in the gel systems ($D_{10} = 0.18$ kGy). *Listeria monocytogenes* ATCC 51414 had a similar resistance in cantaloupes ($D_{10} = 0.15$ kGy) as in the gel systems ($D_{10} = 0.18$ kGy) (Figure 4.14). From these results we can conclude that the selected surrogate was more resistant in cantaloupes compared with the most resistant pathogen. Thus, the gelatin system did not accurately represent the real fruits when prepared and irradiated under these conditions, but the selected surrogate can still be used to indicate decontamination of the target pathogens.

The values of the reduction rate constant (k) observed of the selected surrogate in real cantaloupes ($k = 5.6$ kGy^{-1}) were smaller than the ones obtained in the simulated systems (7 to 13.3 kGy^{-1}), which also suggests that it is easier to kill the surrogate in the real conditions. As expected, for *Listeria monocytogenes* ATCC 51414 the reduction rate was higher ($k = 16.0$ kGy^{-1}) even compared with the rates obtained in the gelatin-systems

	Bacteria strain	Logarithmic reduction (D_{10}) kGy	Coefficient of Variability (CV) %	Linear regression coefficient (R^2)	Reduction rate constant (k) kGy^{-1}
●	*Escherichia coli* K-12 MG1655	0.18[a]	11.291	0.986	11.4
△·	*Listeria monocytogenes* ATCC 51414	0.18[a]	16.874	0.969	13.0
○·	*Escherichia coli* O157:H7 933	0.13[a]	24.110	0.898	15.6
··□··	*Salmonella Poona*	0.13[a]	24.866	0.897	15.9
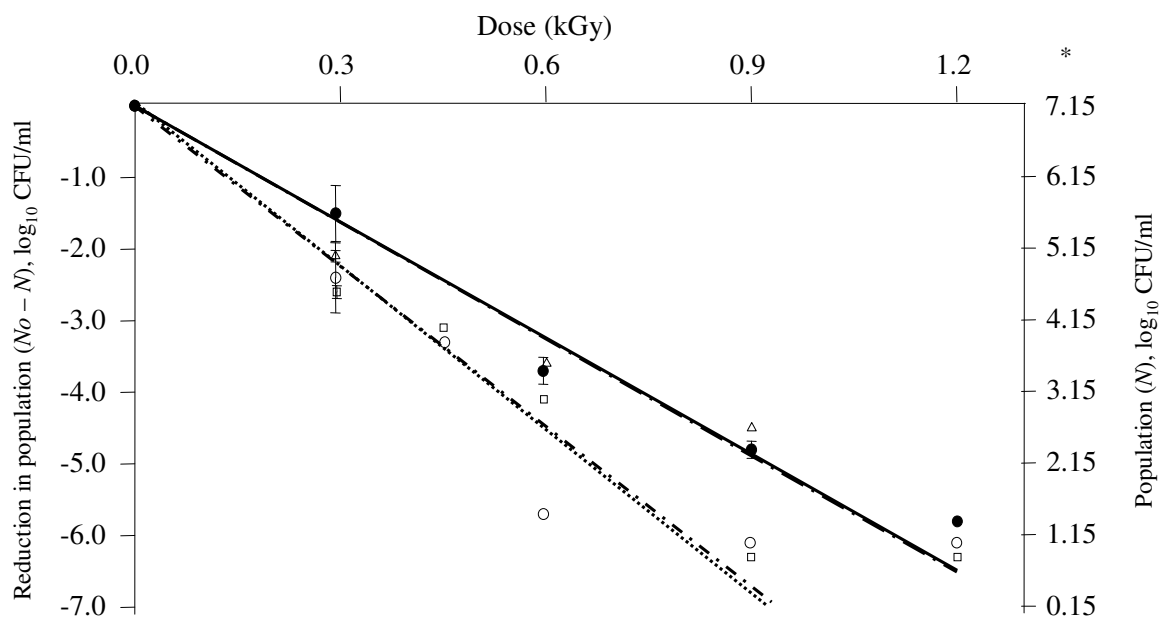
Figure 4.13. Radiation D_{10} values and reaction rate constants (k) for pathogenic strains (open symbols) and the selected surrogate (filled symbol) in gel-based systems.

[a-b] Same letters are mean values statistically similar (p > 0.0001). Samples were irradiated using the *1C25M* configuration (1 cylinder – 0.25 cm height and 6.0 cm diameter, 67.5°, 15.25 cm from beam exit) under a 2.0 MeV Van De Graaff linear accelerator at 20ºC. (n = 5). R^2: Linear regression coefficient is an indicator of the linear relation between two variables. *CV*: Coefficient of Variability is a measure of the variability of a measured parameter expressed relative to the magnitude of the mean. * Average values from four inoculums.

Bacteria strain	Logarithmic reduction (D_{10}) kGy	Coefficient of Variability (CV) %	Linear regression coefficient (R^2)	Reduction rate constant (k) kGy^{-1}
..●.. *Escherichia coli* K-12 MG1655	0.45[a]	5.867	0.994	5.1
–△– *Listeria monocytogenes* ATCC 51414	0.15[b]	11.809	0.981	16.0
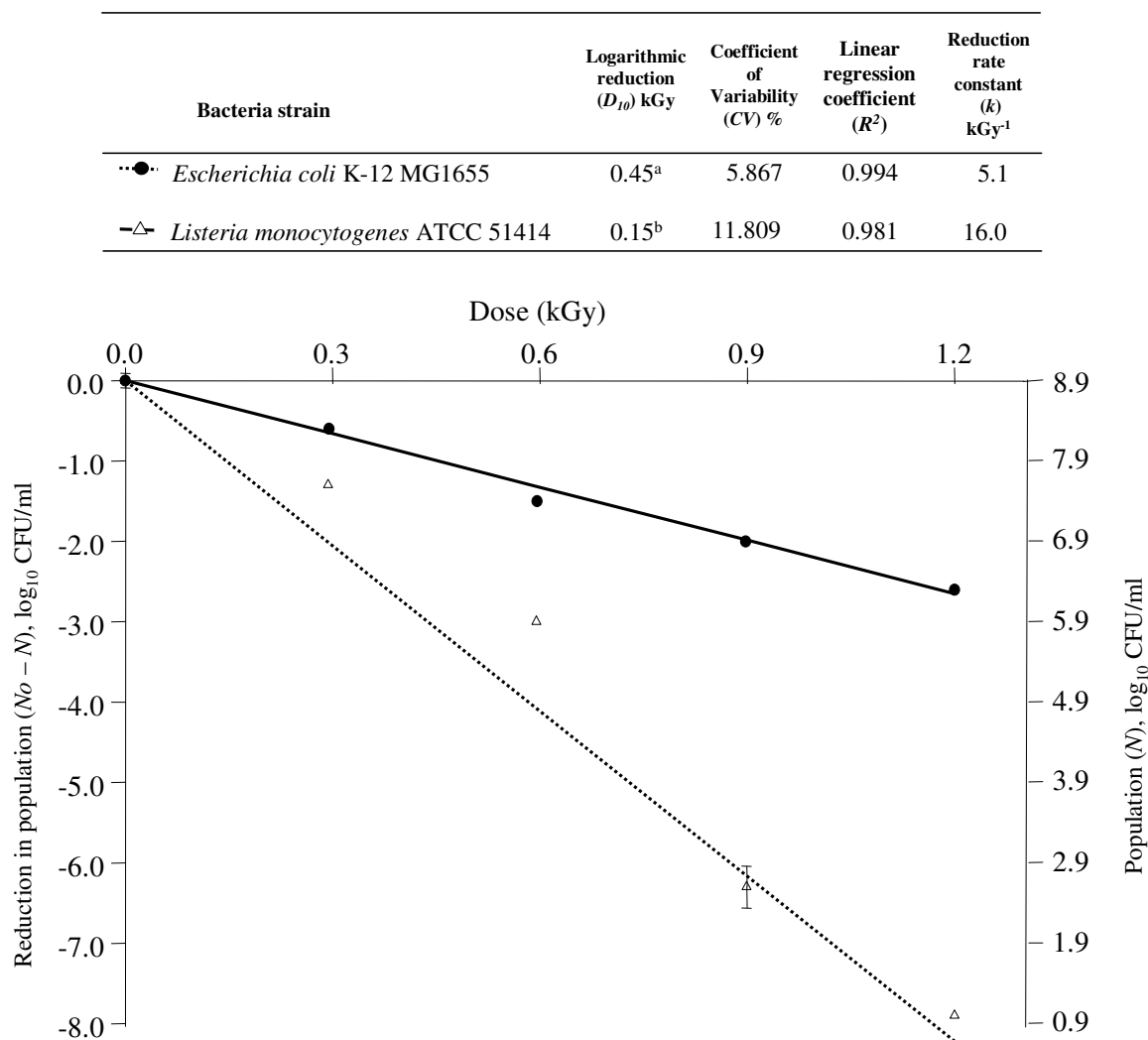
Figure 4.14. Radiation D_{10} values and reaction rate constants (k) for the most resistant pathogen (open symbol) and the selected surrogate (filled symbol) in cantaloupes (*cucumis melo*).

[a-b] Different letters are mean values significantly different (p < 0.0001). Samples were irradiated using the *1C25M* configuration (1 cylinder – 0.25 cm height and 6.0 cm diameter, 67.5°, 15.25 cm from beam exit) under a 2.0 MeV Van De Graaff linear accelerator at 20ºC. 5 replications were made per cylinder. R^2: Linear regression coefficient is an indicator of the linear relation between two variables. *CV*: Coefficient of Variability is a measure of the variability of a measured parameter expressed relative to the mean.

(k = 13.0 kGy^{-1}) and even similar to the obtained in the other pathogens in the same system (15.6 and 15.9 kGy^{-1} for *E. coli* O157:H7 933 and *Salmonella Poona*, respectively).

There are different factors to consider in the validation studies, which may have influenced the results:

1. A model food system cannot reproduce the variability of the real conditions (i. e. pH and microstructure). The effect of the presence of other nutrients (such as proteins, vitamins and minerals) under irradiation treatments and preservation of bacteria were not represented in the gelatin-sugar systems.

2. The behavior of specific strains may differ in different food systems. *Listeria monocytogenes* ATCC 51414 may be affected differently by the radiolytic compounds formed in real cantaloupes, i.e. synthesis and absorption of sugars and the radiolytic compounds resulting from irradiation.

3. The bacteria distribution and mobility within the system may differ in real systems.

Every model system has it limitations; the purpose behind them is to explain one or a few aspects of the real ones. In summary, the gelatin system proved to be an inexpensive, fast method to understand the behavior of different bacteria strains under electron beam irradiation treatments using a 2.0 Van De Graaff linear accelerator.

4.4.2 Physical properties of the model food systems

4.4.2.1 The effect of the addition of sugars

Overall the strength at fracture (σ_{fr}), defined as the force required to destroy the sample, decreased as sugar content increased when the gels were stored at 4°C. Non-irradiated gel systems without sugars (*C*) and those with low sugar content (*ER*) were significantly (p < 0.005) stronger than the irradiated gels without significant changes during the period of study (p < 0.005). Most of the changes were more due to time than due to the treatments in the higher sugar systems (*MR* and *R*). Non-irradiated *MR* systems showed significant (p < 0.005) increase in strength on day 1 (243.0 ± 24.1) and a significant decrease at day 3 (145.8 ± 13.3 kPa) [a decrease of almost 100kPa]. The same decrease was observed in the irradiated systems (70 kPa from day 2 to day 3). The strength of the *R* systems increased during the period of study, showing the maximum strength at day 3 (Table 4.6), probably due to lost of moisture in the sample.

These results suggest that at low temperatures (4ºC) the structure of the gel without sugars (*C*) and with low sugar content (*ER*) may be affected by low dose irradiation (1.0 kGy), while gel systems with high sugar content (*R*) were not affected at all. This may be due to the fact that the structure was not completely set, which was observed during the period of study. Therefore, the system where bacteria populations were inoculated may present different conditions if compared with non-irradiated systems.

Stiffness (modulus of elasticity) decreased with sugar content in the day of irradiation. No significant differences (p < 0.005) were found between irradiated systems

and non-irradiated *C*, *ER* and *MR* systems. The systems with the highest sugar content (*R*) were the least stiff (49.8 ± 6.9 kPa) on the day of irradiation, and their stiffness increased at day 2 (93.9 ± 19.0 kPa) at 4°C, this was especially noted on the irradiated samples (*RI*), which had a 2.5 fold compared to the non-irradiated samples (1.9 fold) (Table 4.6). A decrease in stiffness could be related to a change on microstructure that affects bacteria mobility within the system.

Toughness of the gels significantly ($p < 0.005$) decreased with sugar content (Table 4.6). Statistical differences ($p < 0.005$) were found between irradiated and non-irradiated *C* systems only (Table 4.6). A decrease of toughness with time was observed in the *MR* systems only, the toughness of irradiated systems decreased in the second day, while those non-irradiated until the third day (Table 4.6). This parameter could be used as an indicator of the ratio of water to solids in the sample. Tougher gels may have less free water and thus, more stable.

4.4.2.2 The effects of temperature

Gels were weaker as temperature increased ($p < 0.005$) in all irradiated and non-irradiated samples. No differences were observed between irradiated and non-irradiated samples at 4ºC, while irradiated samples were stronger at higher temperatures.

The treatments stored at 4ºC (*4* and *4I*) and 10ºC (*10* and *10I*) showed a peak in the strength (σ_{fr}) at day 1, while the samples stored at 20ºC (*20* and *20I*) remained without changes (Table 4.7). These results suggest that the structure of the gel prepared under these conditions (gel concentration and pH) is set at low temperatures 48 hours after preparation.

Systems stored at 10ºC were stiffer than those stored at 4ºC and 20ºC (Table 4.7). No significant ($p < 0.005$) differences between irradiated and non-irradiated systems stored at 4ºC were observed in time (Table 4.7), while the irradiated systems stored at higher temperatures (10ºC and 20ºC) were significantly ($p < 0.005$) more elastic (stiffer) than those non-irradiated during the first two days (Table 4.7). Systems stored at 10ºC showed a significant ($p < 0.005$) increase in elasticity at day 1 with a decrease at day 2 and 3, while those stored at 20ºC decreased significantly in elasticity (E) over the period of study (Table 4.7).

It can be concluded that low dose (1.0 kGy) irradiation treatments did not affect the mechanical properties (i. e. structure) of the gels stored at 4ºC. A significant ($p < 0.005$) difference in stiffness between non-treated and treated samples (*MR* and *MRI*) was observed when the storage temperature increased, the development of the structure was still observed at 10ºC while at warm 20ºC temperatures the structure was affected.

Irradiation did not affect the strain at fracture at low temperatures (4ºC). More differences were noticed at the high temperatures. Non-irradiated samples stored at 10°C required more strain to be broken after the second day (3.1 % more), while those irradiated showed differences ($p > 0.005$) in the second day only (Table 4.7). Irradiated samples required more strain to break after the second day when stored at 20°C, while those non-irradiated showed ($p > 0.005$) differences between day 1 and 2 only (Table 3).

The toughness of the samples decreased with temperature. At 10°C significant ($p < 0.005$) differences in toughness between irradiated (*10I*) and non-irradiated (*10*) gels were noticed. The toughness of the irradiated samples stored at 4°C (*4I*) decreased after

Table 4.6

Effect of sugar content (maturity level) on the physical properties of non-irradiated and irradiated (*I*) gel systems stored at 4°C

Maturity Level	Time (days)	Strength (σ_{fr}) kPa		Elasticity (*E*) kPa		Max Strain (ε_{fr}) %		Toughness (kPa)	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Non-sugars irradiated (*CI*)	0	246.1[a]	19.0	97.6[a]	14.4	56.2[a]	2.9	221.5[b]	50.0
	1	253.8[a]	19.7	100.1[a]	9.1	54.4[a]	1.5	204.5[b]	14.2
	2	230.2[a]	16.1	113.3[a]	16.9	52.9[a]	1.2	174.9[a]	12.3
	3	296.8[a]	12.9	106.9[a]	13.4	56.9[a]	2.4	250.6[c]	11.5
Non-sugars (*C*)	0	299.0[a]	16.9	104.5[a]	22.1	62.5[a]	1.9	294.4[b]	22.9
	1	265.0[a]	28.7	93.7[a]	13.2	60.8[a]	1.2	284.8[b]	22.1
	2	262.4[a]	25.2	91.6[a]	16.4	61.2[a]	2.0	266.3[a]	7.4
	3	331.8[a]	27.3	109.7[a]	9.7	59.2[a]	1.8	333.7[c]	16.3
Early Ripe Irr.(*ERI*)	0	220.2[a]	25.1	62.3[a]	5.6	65.1[a]	1.2	238.7[a]	33.2
	1	215.5[a]	20.5	63.6[a]	5.7	64.4[a]	1.3	223.5[a]	20.4
	2	226.7[a]	4.5	76.2[a]	6.7	62.0[a]	-	171.2[b]	5.2
	3	179.7[a]	9.5	70.0[a]	21.6	60.2[b]	3.8	190.1[a]	17.1
Early Ripe (*ER*)	0	239.2[a]	3.9	88.6[a]	8.0	53.1[a]	0.7	229.1[a]	41.7
	1	226.7[a]	15.3	82.9[a]	-	57.6[a]	-	239.1[a]	34.6
	2	233.6[a]	13.4	77.7[a]	14.3	57.4[a]	1.7	217.8[a]	26.7
	3	207.7[a]	42.8	63.5[a]	21.4	65.1[a]	1.2	213.7[a]	36.4
Mod. Ripe Irradiated (*MRI*)	0	220.0[a]	9.9	67.9[a]	5.0	64.7[a]	1.5	221.9[a]	14.8
	1	252.9[a]	24.1	86.5[a]	26.1	61.0[a]	3.0	257.5[a]	37.8
	2	221.5[a]	35.3	78.2[a]	12.0	61.5[a]	-	169.8[b]	31.2
	3	151.0[b]	3.8	67.0[a]	12.7	62.7[a]	2.9	141.2[b]	18.4
Mod. Ripe (*MR*)	0	203.8[b]	14.5	67.2[a]	3.2	63.2[a]	3.0	198.0[b]	26.6
	1	243.0[a]	24.1	80.1[a]	14.9	62.1[a]	1.7	254.6[a]	16.0
	2	205.8[b]	17.3	67.3[a]	14.1	63.1[a]	2.9	219.2[b]	14.5
	3	145.8[c]	13.3	73.0[a]	1.9	64.1[a]	1.7	165.0[c]	30.1
Ripe Irradiated (*RI*)	0	168.0[c]	2.4	58.4[b]	4.8	59.3[a]	1.4	165.2[a]	12.2
	1	217.9[b]	30.6	87.9[b]	20.5	58.5[a]	1.1	208.8[a]	16.5
	2	243.9[ab]	7.5	141.0[a]	8.0	51.6[c]	1.5	232.1[a]	57.4
	3	281.7[a]	27.6	147.2[a]	24.6	54.9[b]	1.3	194.5[a]	24.7
Ripe (*R*)	0	141.0[c]	13.4	49.8[b]	6.9	63.2[a]	2.7	160.3[a]	27.0
	1	195.7[b]	26.0	49.3[b]	7.8	66.5[a]	3.4	204.1[a]	31.5
	2	229.8[a]	7.5	93.9[a]	19.0	54.9[b]	3.1	193.0[a]	28.5
	3	241.4[a]	7.4	82.8[a]	21.6	61.4[a]	5.1	188.1[a]	12.3

[a-c] Different letters are mean values significantly different (p < 0.005), n = 5. (Appendix D1 to D16). Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV linear accelerator (20°C). Texture analysis was made in a TA.XT2 Texture Analyzer at 0.5 mm/s and 0.05 N. *C*: no-sugars, *ER*: 3 %, 1.5:1.5:0, *MR*: 5.5 %, 1:1:1, *R*: 8 %, 0.5:0.5:2 (% sugars w/v, glucose: fructose : sucrose). *SD*: Standard Deviation.

Table 4.7

Effect of the temperature on the physical properties of non-irradiated and irradiated (*I*) moderately ripe (*MR*) systems

Storage temperature (°C)	Time (days)	Strength (σ_{fr}) kPa		Elasticity (*E*) kPa		Max Strain (ε_{fr}) %		Toughness (kPa)	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
4I	0	220.0[a]	9.9	67.9[a]	5.0	64.7[a]	1.5	221.9[a]	14.8
	1	252.9[a]	24.1	86.5[a]	26.1	61.0[a]	3.0	257.5[a]	37.8
	2	221.5[a]	35.3	78.2[a]	12.0	61.5[a]	-	169.8[b]	31.2
	3	151.0[b]	3.8	67.0[a]	12.7	62.7[a]	2.9	141.2[b]	18.4
4	0	203.8[b]	14.5	67.2[a]	3.2	63.2[a]	3.0	198.0[b]	26.6
	1	243.0[a]	24.1	80.1[a]	14.9	62.1[a]	1.7	254.6[a]	16.0
	2	205.8[b]	17.3	67.3[a]	14.1	63.1[b]	2.9	219.2[b]	14.5
	3	145.8[c]	13.3	73.0[a]	1.9	64.1[a]	1.7	165.0[c]	30.1
10I	0	186.1[b]	11.0	92.8[b]	16.0	60.1[a]	1.2	171.1[b]	13.6
	1	245.2[a]	11.4	143.2[a]	6.4	57.7[b]	1.2	227.9[a]	23.7
	2	173.1[b]	13.5	63.7[c]	15.6	62.3[a]	2.7	154.8[b]	27.8
	3	150.3[c]	17.7	71.2[c]	13.1	60.8[a]	1.5	160.9[b]	10.9
10	0	129.4[bc]	31.1	66.1[b]	33.5	60.8[ab]	3.2	131.2[a]	20.3
	1	176.5[a]	14.3	109.0[a]	20.1	57.8[bc]	1.5	161.1[a]	19.8
	2	155.4[ab]	26.7	63.8[b]	2.0	62.1[a]	1.9	159.6[a]	26.0
	3	118.1[b]	19.2	72.5[b]	18.6	55.8[c]	2.5	100.3[b]	14.4
20I	0	171.4[a]	21.5	89.4[a]	25.8	60.0[c]	1.9	138.4[a]	17.0
	1	142.6[a]	8.8	68.7[ab]	8.1	60.3[c]	1.4	120.7[a]	10.2
	2	157.1[a]	7.0	62.1[ab]	8.0[a]	62.7[b]	0.7	136.0[a]	6.7
	3	159.3[a]	31.4	48.0[b]	6.6	64.8[a]	1.9	125.1[a]	21.7
20	0	149.5[a]	17.1	70.6[a]	8.6	61.1[b]	1.0	133.3[a]	17.0
	1	128.8[a]	15.3	38.2[c]	5.3	64.4[a]	2.1	111.9[a]	14.1
	2	147.7[a]	4.3	56.6[bc]	10.4	61.9[ab]	2.0	121.6[a]	5.4
	3	142.1[a]	15.8	48.0[c]	4.4	63.8[ab]	1.6	128.0[a]	17.5

[a-c] Different letters are mean values significantly different (p < 0.005), n = 5. (Appendix D17 to D24). Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). *MR*: 5.5% w/v total sugars (1:1:1, glucose:fructose:sucrose) Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. *SD* = Standard Deviation.

the second day while those stored at 10°C (*10I*). Those systems stored at 20°C (*20I* and

20) showed no changes in toughness (Table 4.7).

The time to melt the gel cylinders, in the incubator at 37°C, varied between 16

and 26 minutes with no significant differences ($p > 0.005$) due to sugar content or

storage time. The density of the gels was close to that of water, ranging between 961 and

1030 kg /m^3 without significant ($p > 0.005$) changes in the period of study. The water

activity (Aw) of the samples ranged between 0.94 and 0.96 with a tendency to increase

during the period of study. A more accurate method of measurement may detect

differences in these properties among the treatments.

4.4.3 Bacteria distribution in the gel systems

4.4.3.1 Microbial analysis

Escherichia coli K-12 MG1655 was found in proportional amounts (6.6 $\log_{10}$

CFU/ml or 25%), in the four (4) slices of the gelatin cylinders after 2.5 hours while in

agar it took around 2 hours (Figures 4.15 to 4.18). Significant ($p < 0.005$) differences

were encountered between treatments (gel and agar). This suggests that the bacteria

when left at room temperature (20°C) in a gel-like food system (without sugars) moves

within the system at a rate of approximately 0.4 cm/h to 0.5 cm/h (gelatin and agar

respectively). It should be considered that this penetration rate applies for the specific

settings of the design (gel concentration, amount of inoculum, sample size and design

and temperature).

It was determined that in the top (*T*) layer, first 0.25 cm from the surface, the

population (*P*) decreases every hour in gelatin and agar systems in almost 51.1 % and 67.1 % per hour during the first hour respectively (Figure 4.15). Stability in numbers (25%) was achieved after the first hour. On the other hand, during the first hour on the medium top layer (*MT*), *P* increases in almost 17.6 % and 25.5 % per hour (Figure 4.16), in the medium bottom (*MB*) 16.5 % and 23.0 % (Figure 4.17), and bottom (*B*) 17.0 %, 18.6 % (Figure 4.18) for gelatin and agar respectively. These results suggest that the bacteria have more difficulty penetrating the agar compared to the gelatin-based system, which may have a more porous structure. In addition, the increase in population in the bottom (*B*) layer is slower probably due to a decrease in the pressure for migration. Among the factors to be considered for this study is the sample and inoculum size, the geometry of the system and the temperature. Bigger samples or smaller inoculum may decrease the penetration, and it has been suggested that flat or convex surfaces allow a faster movement of macroparticles (Guinee and Fox, 1993) in gels making it easier for bacteria to penetrate the system. Warmer temperatures influence the structure of the gels and therefore the free water (Aw), which allows bacteria to swim.

4.4.3.2 Microscopy analysis

In the period of study (3 hours) it was observed that the density (ρ_b) of *E. coli* K-12 MG1655 in the top of the gel increased almost 30 times due to concentration of bacteria in the samples. As the samples dried the average distance traveled (*d*) decreased almost by a half, the number of swimmers (S_w) by 90 times and their velocity (*v*) by 3 times (Table 4.8). The velocity of movement (*v*) correlated well ($R^2 = 0.708$) with the

area of movement ($\overline{A}$) (Figure 4.19), which suggests that a single bacterium not only slows its movement, but also is restricted in the area. These results are represented in Figures 4.20 to 4.22 where the movement of bacteria was traced and presented in a grid format. The decrease in movement of *E. coli* K-12 MG1655 was due to drying of the surface water of the gelatin system and to the vertical (depth) penetration of the microbes in the gelatin.

One of the advantages of using the gelatin system is their translucency, or capacity to transmit light trough smaller thickness. When observed within the first 0.6 mm under the microscope, no significant differences ($p < 0.001$) were found in the proportion of bacteria within the gel after 3 hours of inoculation (Figure 4.23). After 1 and 2 hours of inoculation (Figure 4.23) a high percentage of the population (60 and 30%) was still on the top 0.065 mm.

When bacteria were mixed with the gel to represent the ideal distribution (Figure 4.24), no statistical differences were detected ($p = 0.03$). Within the limitations of the two methods (Table 4.9) the results were in agreement with those obtained in Section 4.4.3.1.

These results support the hypothesis that bacteria may migrate throughout a system which may be either porous or have a high water content. This knowledge is of importance when designing irradiation treatments where the penetration depth is limited. Among the factors that influence bacteria migration are: gravity pressure, osmosis gradient, decrease in availability of nutrients, presence/absence of oxygen.
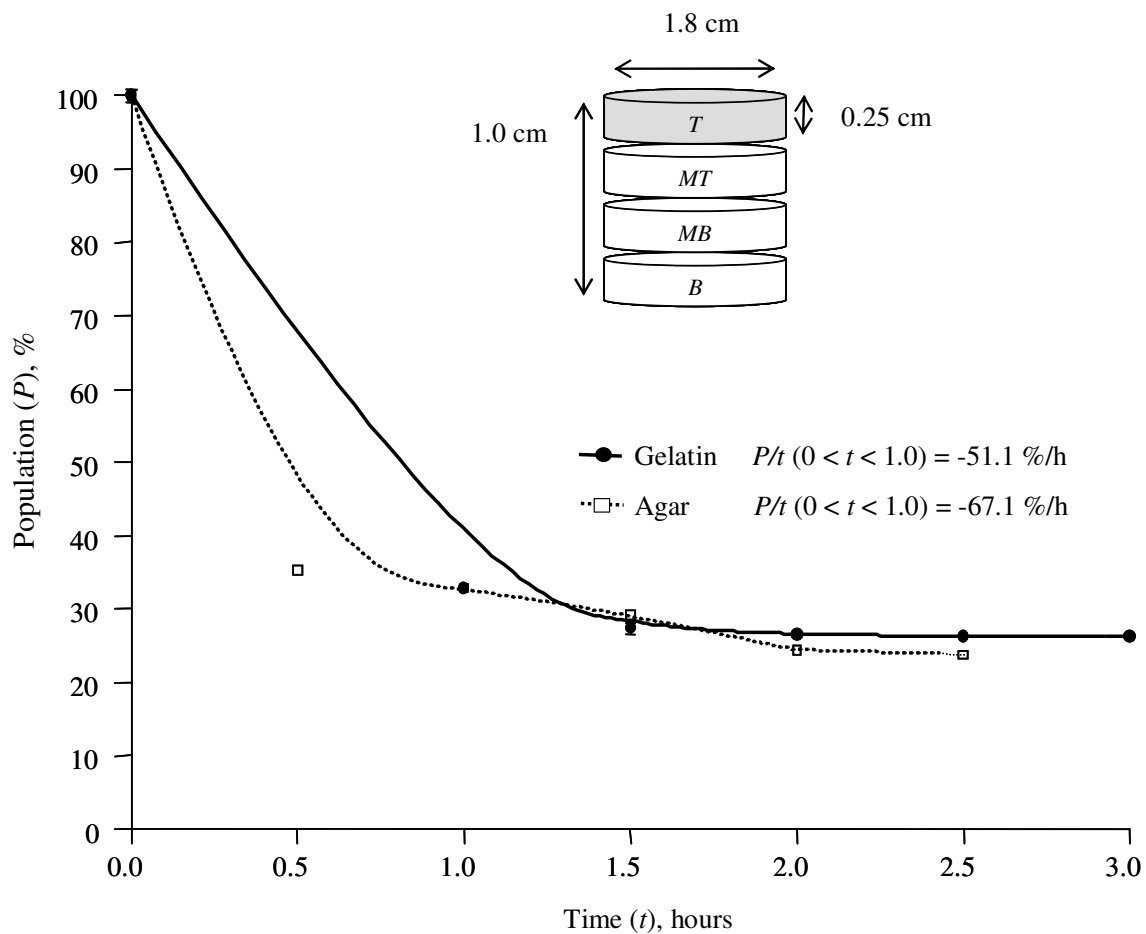
Figure 4.15. Distribution of *Escherichia coli* K-12 MG1655 in the top (*T*) Layer (0 to 2.5 mm) after uniaxial inoculation in the top of gelatin and agar cylinders (*D* = 1.8 cm, *h* = 1.0 cm) at room temperature (20°C), evaluated by standard plate counts (n = 3).
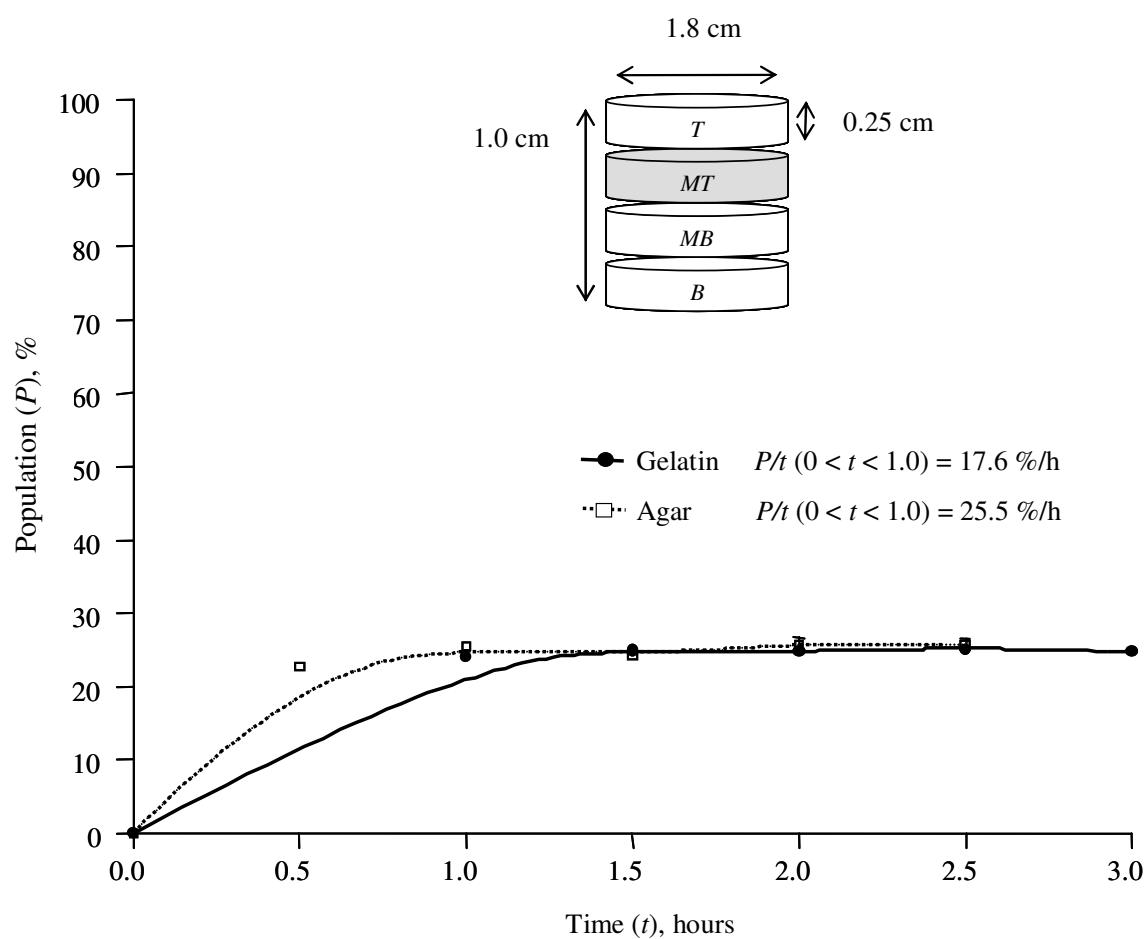
Figure 4.16. Distribution of *Escherichia coli* K-12 MG1655 in the medium top (*MT*) Layer (2.5 to 5.0 mm) after uniaxial inoculation in the top of gelatin and agar cylinders (*D* = 1.8 cm, *h* = 1.0 cm) at room temperature (20°C), evaluated by standard plate counts (n = 3).
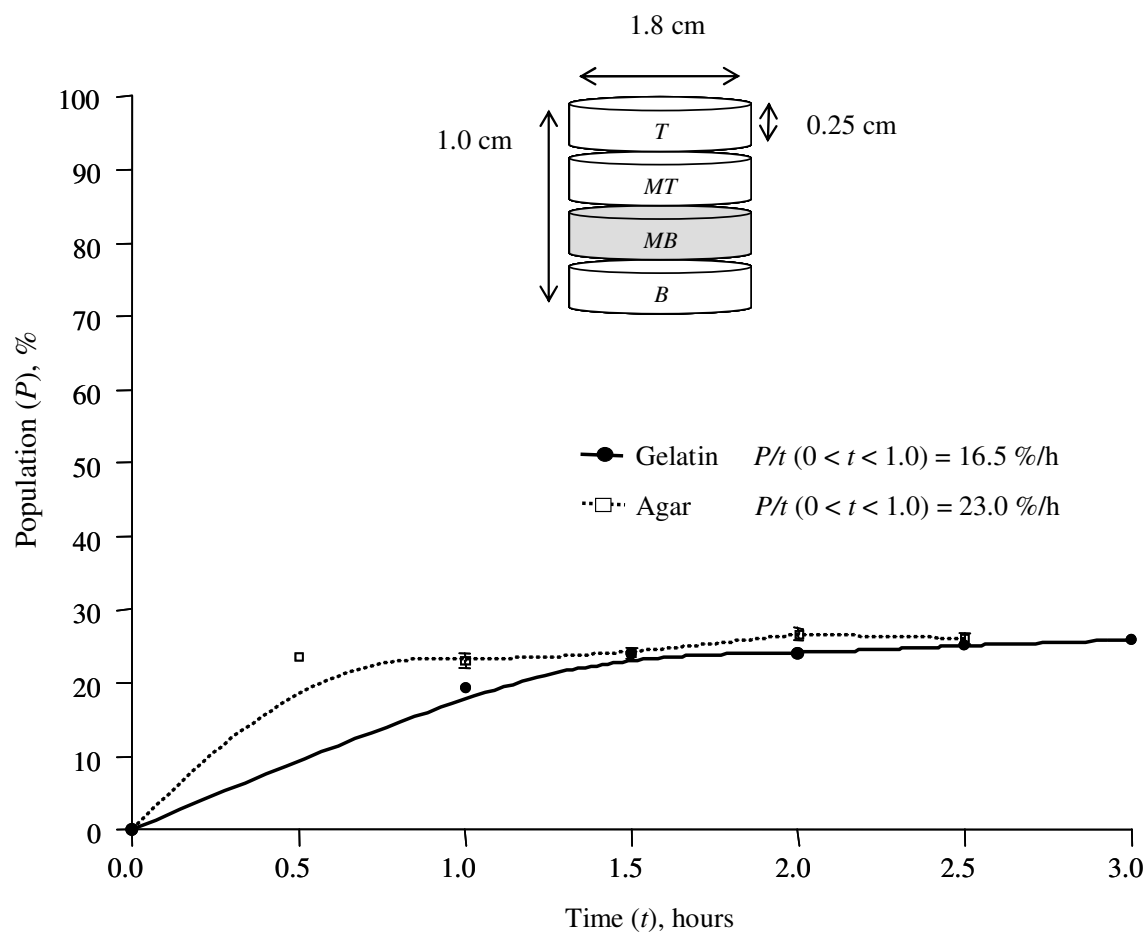
Figure 4.17. Distribution of *Escherichia coli* K-12 MG1655 in the medium bottom (*MB*) Layer (5.0 to 7.5 mm) after uniaxial inoculation in the top of gelatin and agar cylinders ($D = 1.8$ cm, $h = 1.0$ cm) at room temperature (20°C), evaluated by standard plate counts (n = 3).
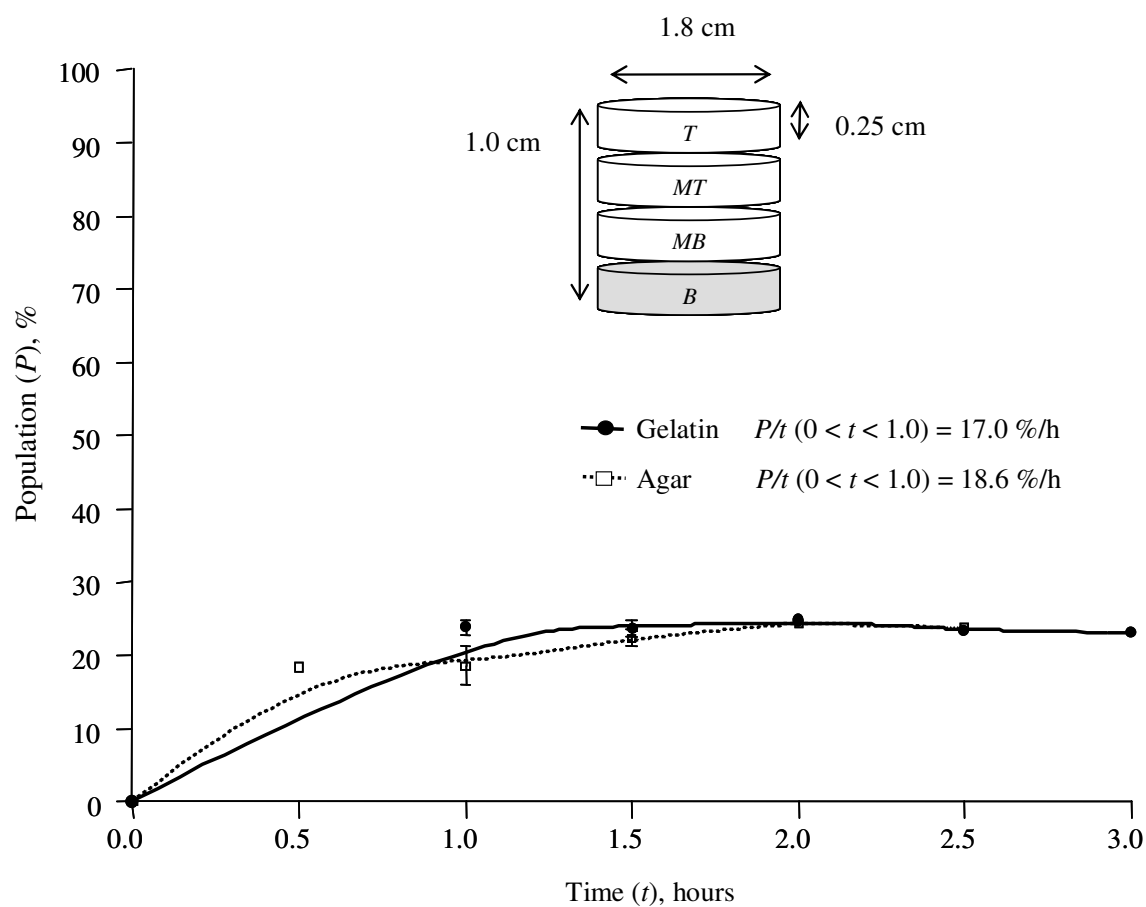
Figure 4.18. Distribution of *Escherichia coli* K-12 MG1655 in the bottom (*B*) Layer (7.5 to 10.0 mm) after uniaxial inoculation in the top of gelatin and agar cylinders (*D* = 1.8 cm, *h* = 1.0 cm) at room temperature (20°C), evaluated by standard plate counts (n = 3).

Table 4.8

Indicators of horizontal displacement of *Escherichia coli* K-12 MG1655 in the surface (horizontal axis) of gelatins

Time after inoculation (t), hours	1	2	3
Bacteria density (ρ_b), Bacteria/100 μm^2	0.2	6.0	7.1
Swimmers (S_w), %	90.5	0.4	1.4
Time of measurement (t_m), s	11	11	16
Distance traveled (d), μm	107.8 ± 160.8	62.9 ± 41.5	46.1 ± 26.8
Velocity (v), μm/s	10.1 ± 15.3	6.0 ± 3.8	2.9 ± 2.0
Area of movement ($\overline{A}$), μm^2	373.8 ± 669.1	82.2 ± 127.3	693.8 ± 1199.4

Measurements were made in pixels using a Java Application (ImageJ. 1.33u) to process digital pictures taken in a light microscope with a magnification of 20X to inoculated gelatin layers (0.25 x 3.75 x 0.1 cm) at room temperature 20˚C at a frame rate of 0.4 to 0.6 frames per second.

$$v = 0.03901\,\overline{A} + 1.78128$$
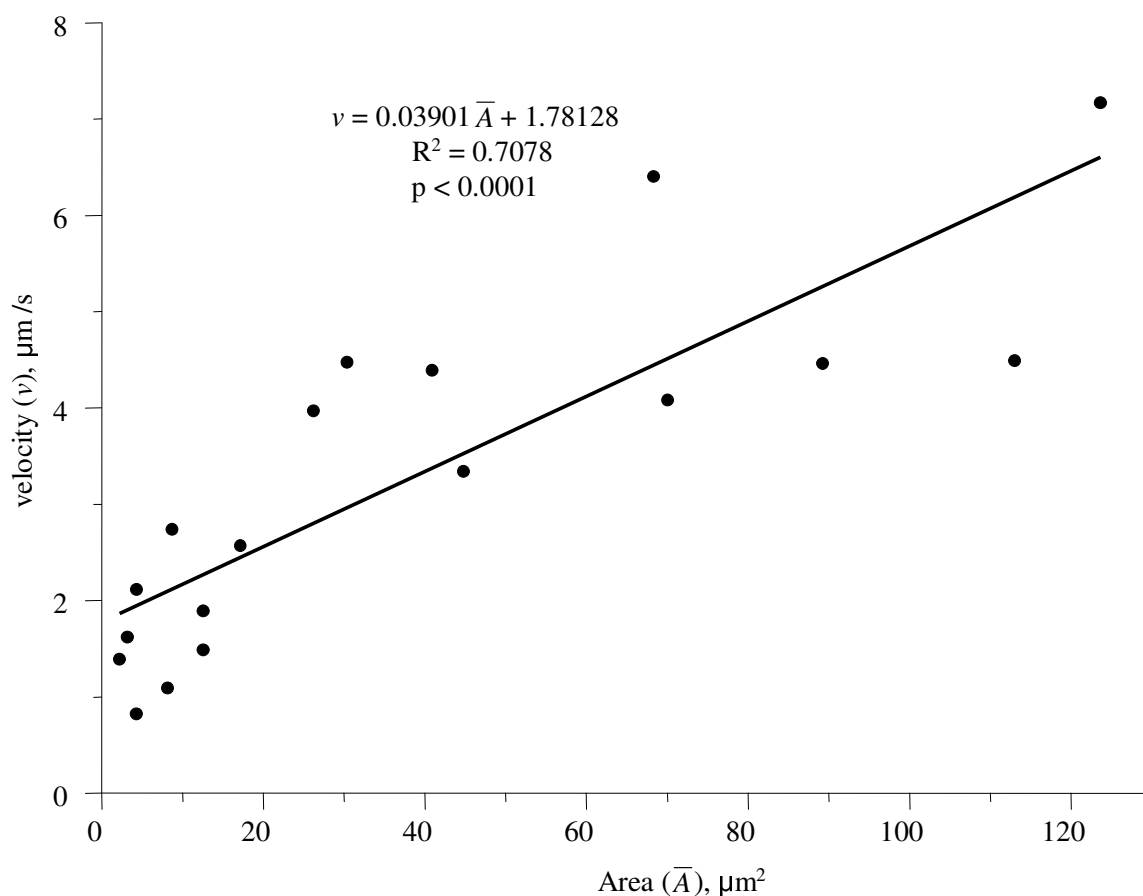$$R^2 = 0.7078$$
$$p < 0.0001$$

Figure 4.19. Relationship between velocity of displacement of microbes and traveled area in gelatin (10 % w/v) systems using *Escherichia coli* K-12 MG1655 at 20°C.

Results shown were calculated from measurements made in pixels using a Java Application (ImageJ. 1.33u) to process digital pictures taken in a light microscope with a magnification of 20X to inoculated gelatin layers (0.25 x 3.75 x 0.1 cm) at room temperature 20˚C at a frame rate of 0.4 to 0.6 frames per second.

Figure 4.20. Horizontal displacement of *Escherichia coli* K-12 MG1655 in the surface of gelatin cylinders (10 % w/v) after 1 hour of inoculation at 20°C.

Photographs were taken at intervals 0.4 seconds per frame; the points represent the reference point in the computer monitor. Measurements were made in pixels using a Java Application (ImageJ. 1.33u) to process digital pictures taken in a light microscope with a magnification of 20X to inoculated gelatin layers (0.25 x 3.75 x 0.1 cm).
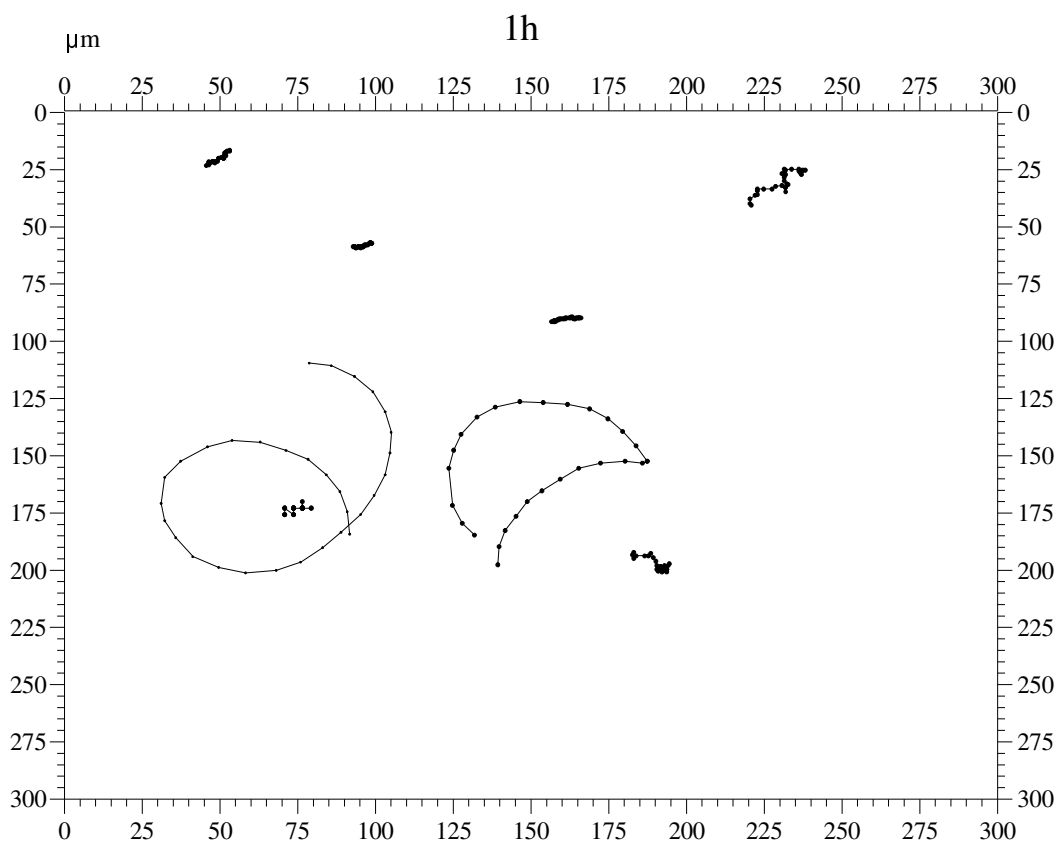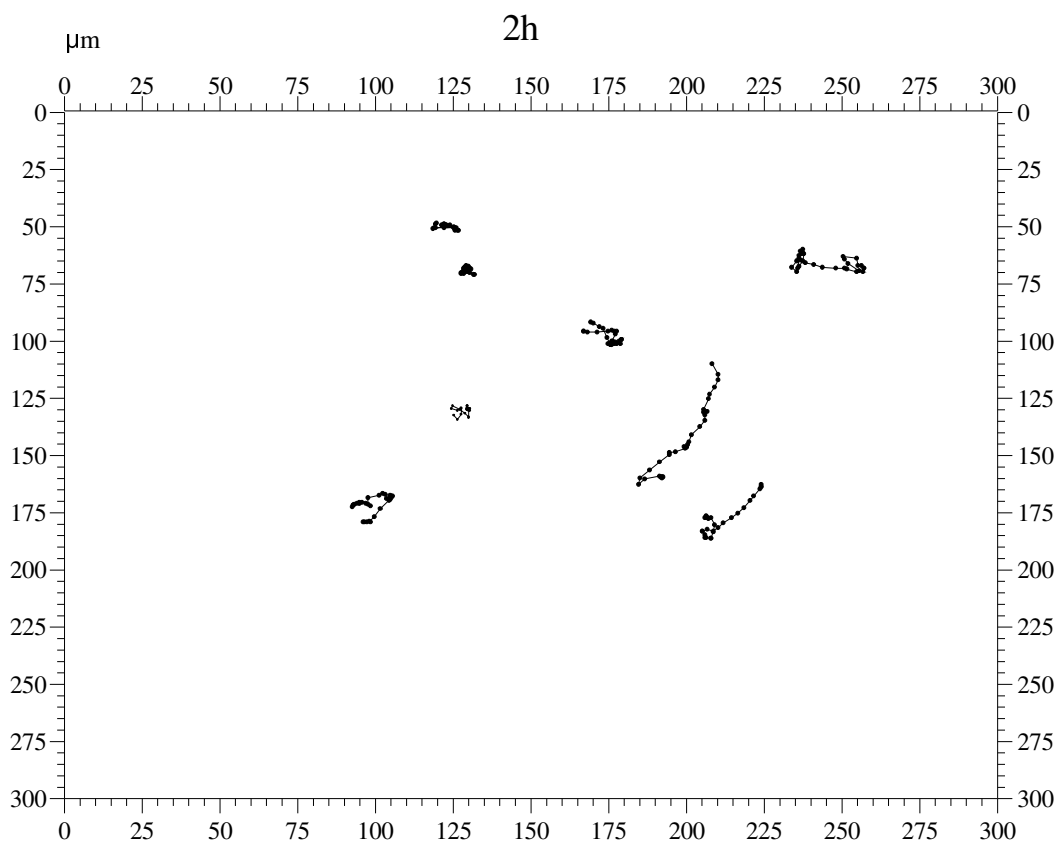
Figure 4.21. Horizontal displacement of *Escherichia coli* K-12 MG1655 in the surface of gelatin cylinders (10 % w/v) after 2 hours of inoculation at 20°C.

Photographs were taken at intervals 0.4 seconds per frame; the points represent the reference point in the computer monitor. Measurements were made in pixels using a Java Application (ImageJ. 1.33u) to process digital pictures taken in a light microscope with a magnification of 20X to inoculated gelatin layers (0.25 x 3.75 x 0.1 cm).

Figure 4.22. Horizontal displacement of *Escherichia coli* K-12 MG1655 in the surface of gelatin cylinders (10 % w/v) after 3 hours of inoculation at 20°C.

Photographs were taken at intervals 0.6 seconds per frame; the points represent the reference point in the computer monitor. Measurements were made in pixels using a Java Application (ImageJ. 1.33u) to process digital pictures taken in a light microscope with a magnification of 20X to inoculated gelatin layers (0.25 x 3.75 x 0.1 cm).
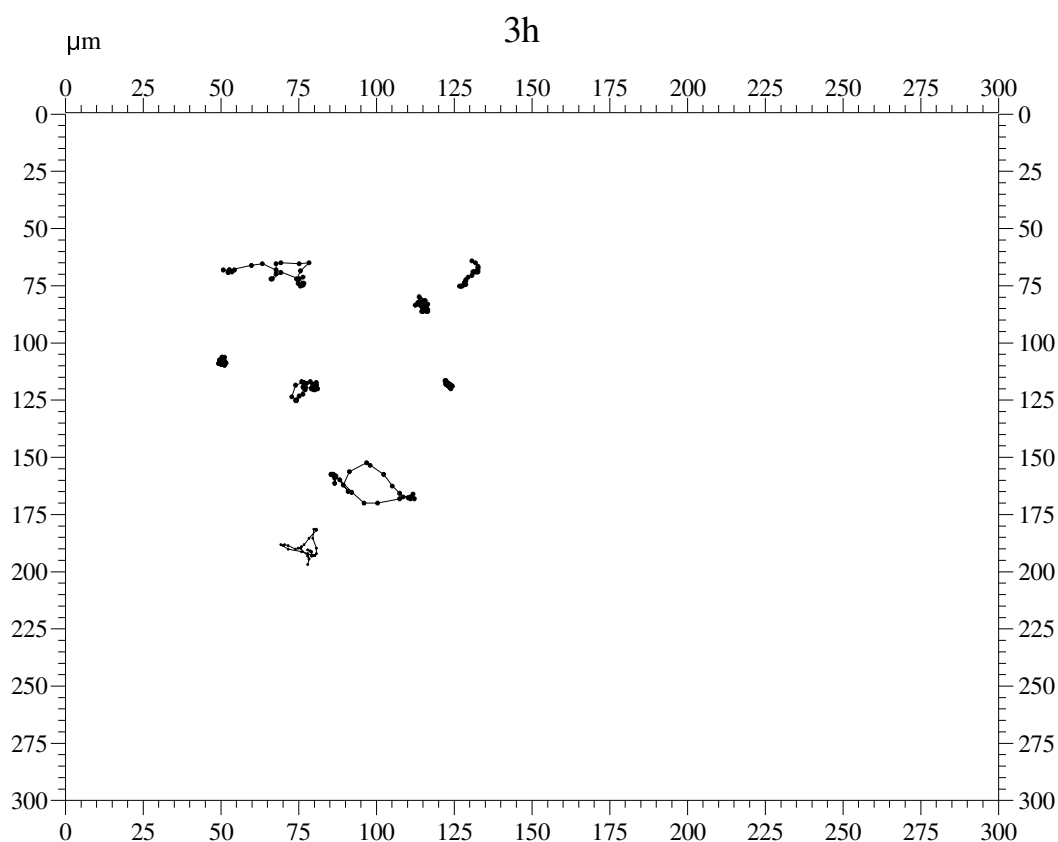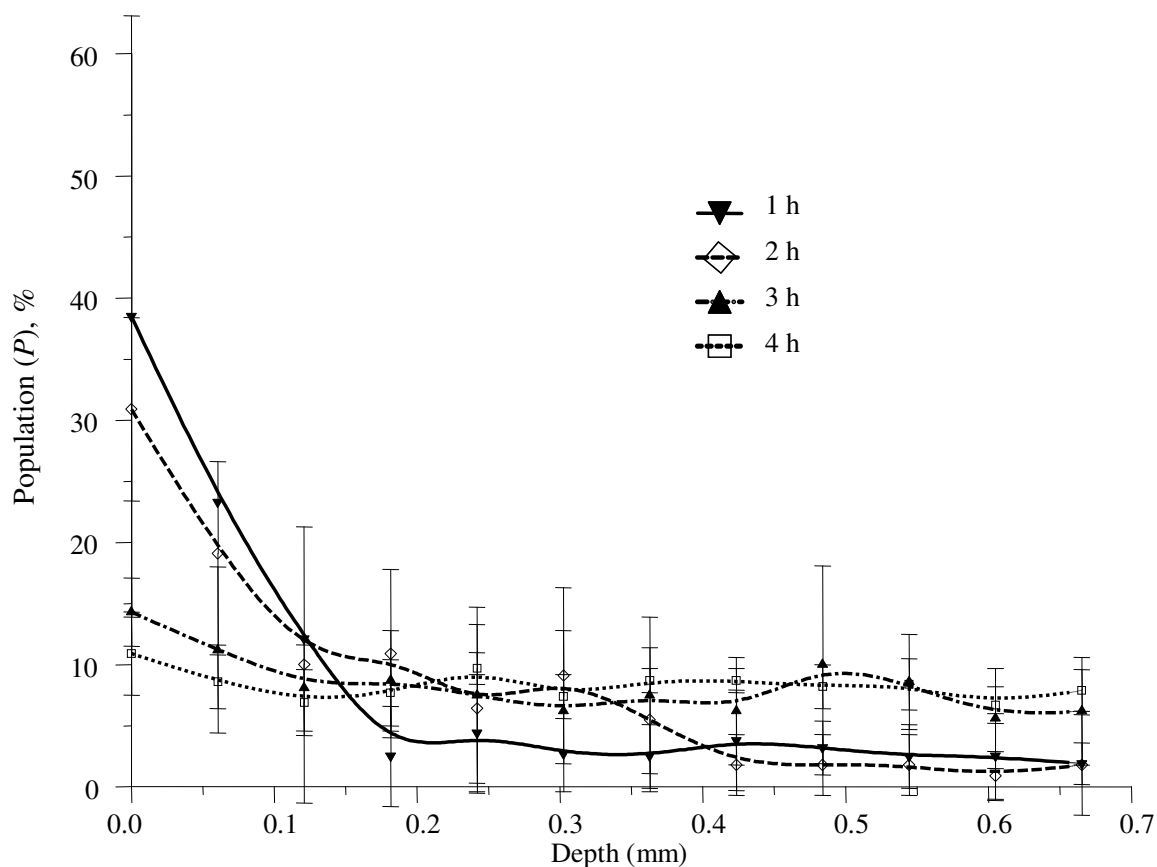
Figure 4.23. Distribution of bacteria (*Escherichia coli* K-12 MG1655) within the top 0.6 mm of the gel system evaluated using microscope techniques during a 4 hours period after inoculation at room temperature (20°C).

Photographs were taken at intervals 0.0605mm, bacteria was counted visually by grayscale differences. Digital pictures were analyzed using a Java Application (ImageJ. 1.33u) originally acquired in a light microscope with a magnification of 10X from inoculated gelatin cylinders of 1.8 cm diameter x 0.4 cm height.

Table 4.9

Comparison of standard plate counts and microscope techniques

	Method	
	Sample dissection and platting	**Microscope evaluation**
Sample preservation	It is difficult to achieve a smaller thickness of the layers and to dissect the sample without altering the microbial population.	Sample is conserved.
	Variability of vertical distribution within one sample is difficult to measure.	Bacteria distribution can be traced vertically in the same spot.
Measurements	Bacteria numbers rely on bacteria capable to recover and form a colony.	Real Time, and total numbers of microbes (alive and death), which accounts for movement due to gravity/osmosis.
	Smaller numbers of bacteria are difficult to count (concentration of bacteria is needed).	Lower densities of bacteria can be counted.
	The whole sample can be evaluated.	Measurements are made in smaller areas of the sample (more variability) and the depth is limited.
	Standard method.	Correlates with standard method, but not approved.
Future applications	Killed populations cannot be measured using this technique.	Evaluation of damage and killing could be evaluated in the micro scale by the application of staining techniques.
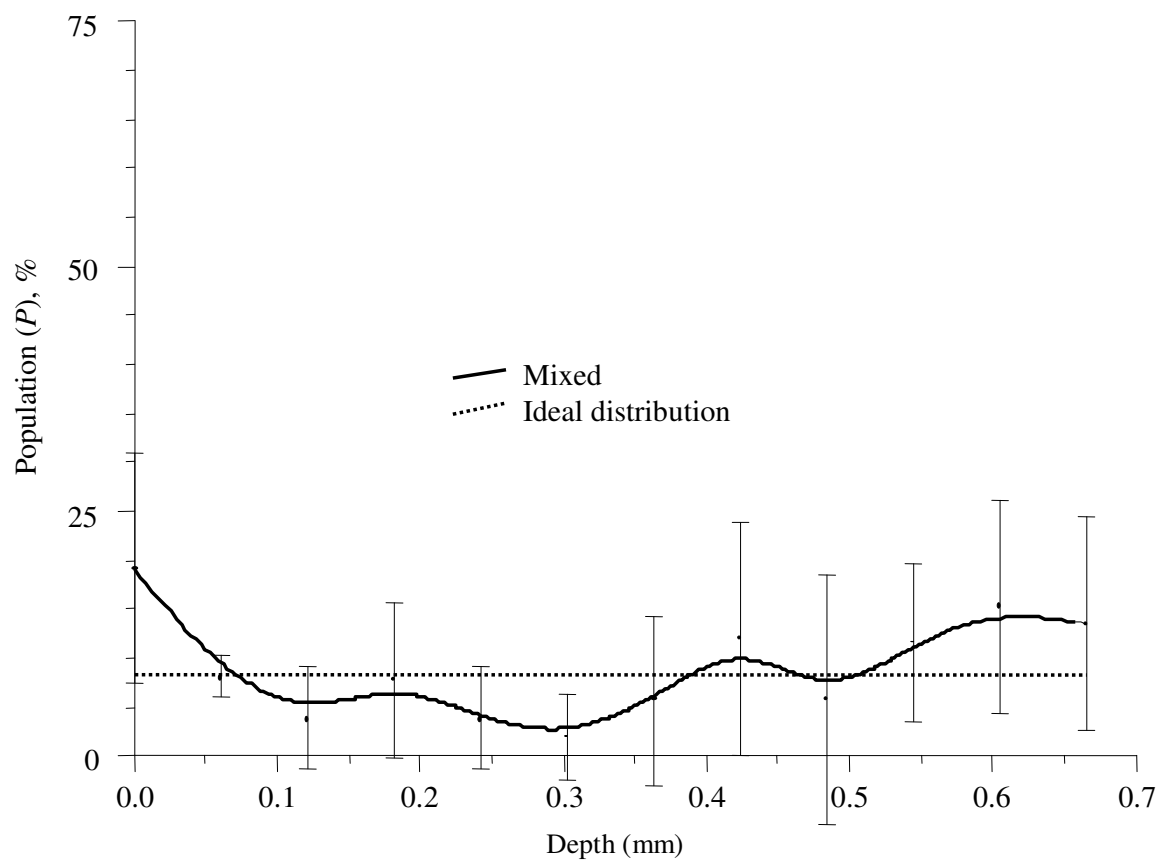
Figure 4.24 Evaluation of bacteria populations mixed in the gel system during preparation using the microscope method.

Photographs were taken at intervals 0.0605mm, bacteria was counted visually by grayscale differences. Digital pictures were analyzed using a Java Application (ImageJ. 1.33u) originally acquired in a light microscope with a magnification of 10X from gelatin cylinders (1.8 cm diameter x 0.4 cm height) mixed with inoculum prior setting at 4°C.

CHAPTER V

SUMMARY AND CONCLUSIONS

Because the purpose of this study was to measure the different parameters that affect the application of electron beam irradiation and fruit maturity stage (sugar content) in the effectiveness of this technology to reduce populations of bacteria surrogates and their ability to recover, several conclusions can be made.

A preliminary study with gelatin-based systems under electron beam irradiation treatments demonstrated that *Escherichia coli* K12 MG1655 is a suitable surrogate of the three most common pathogens encountered in fresh produce.

The gelatin systems proved to be inexpensive and easy to prepare for use in the evaluation of the potential surrogates.

The penetration depth of the 2.0 MeV Van de Graaff linear accelerator was determined to be 0.25 cm by analyzing the killing of microbes in four layers of gelatin-based cylinders using standard plate count methods. This result was confirmed using Monte Carlo simulation methods to predict dose distribution. A coverage area of 6.0 cm in diameter was also determined when the sample was placed 15.25 cm (6.0 inches) away from the beam in a perpendicular position. These preliminary studies explained the relative large radiation D_{10} values obtained in the first configuration (*5C1* = 5 cylinders – 1.0 cm height x 1.78 cm diameter, 22.5°, 30.5 cm from the beam exit). Radiation D_{10} values for the selected surrogate (*Escherichia coli* K-12 MG1655) and the three pathogens (*Listeria monocytogenes* ATCC 51414, *Escherichia coli* O157:H7 933 and *Salmonella Poona*) obtained using the optimum configuration (*1C25M* = 1 cylinder –

0.25 cm height x 6.0 cm diameter, 67.5°, 15.25 cm from the beam exit) were comparable with those reported in the literature. This result confirms that accuracy in dose measurement and dose distribution to ensure a uniform dose is achieved is critical to improve future irradiation experiments.

The variation in the sugar content of the medium did not show a significant effect on the effectiveness of the irradiation treatments as a decontamination technology. Therefore, the sugar content has not a factor in the gelatin systems, and a study with higher sugar concentrations or using a different medium (i. e. water) where single sugars are irradiated may demonstrate different results. If these results can be reproduced in real food systems, it will improve the radiation treatments from the microbiological point of view, because the ripeness of fruits will not be a limiting factor.

A recovery of all irradiated microbial populations to their initial numbers was observed after one day of inoculation at room temperature (20ºC). These results were confirmed in the modified design where the generation times (*GT*) were almost the same as those for non-irradiated samples regardless of the maturity level. To prevent recovery of bacteria populations after irradiation treatments, storage at low temperatures (4ºC) is recommended. Samples stored at this low temperature maintained low population numbers up to 4 days regardless of sugar content.

The resistance of the selected surrogate to irradiation was successfully validated in the modified gel system design and with real cantaloupes. The potential of this selected strain is unlimited, not only because of its resistance to irradiation, but because its genome sequence has been identified and the production of mutants is feasible.

In general, irradiation at 1.0 kGy only caused a few changes of the four measured physical properties of the gels. Most of the changes were due to storage temperature and time, which are important to understand because they could be related to changes in the gel structure, and therefore to the availability of nutrients for recovery of microbes. The relative low dose used in this study and its distribution explain these results.

Vertical movement in one axis (z axis) of the selected surrogate within the gels was confirmed using microbial counts. Microscope techniques were used to understand the movement of bacteria in three dimensions. Both methods showed similar results within their advantages and limitations. Overall, time (1 hour) and depth (0.6 mm) are factors that decreased the movement of bacteria. Some potential applications include: the use of inoculated gelatins to determine the dose achieved within the sample and the application of gel stains to determine the distribution of damaged bacteria in translucent gels.

CHAPTER VI

RECOMMENDATION FOR FURTHER STUDIES

The recommendations for further studies can be listed based on the scientific disciplines applied in this study: applied microbiology, the application of electron beam irradiation to food systems, the study of the structure of gels and microscopy.

Due to the natural variability of the bacteria strains future research should focus on a particular strain with characteristics that have been already studied and explained in detail. Modifications to the strain (i. e., creating mutant resistant populations by subjecting to subsequent radiation treatments) may allow a better understanding of microbial damage and recovery. Different bacteria species recover differently depending on their mechanisms to adapt to stress.

Dose measurement and distribution (mapping) within the sample using living organisms (indicators) and staining and novel microscope techniques (biological dosimeters).

The use of other translucent gel systems where injured bacteria can be observed could improve the use of a model system in food irradiation research. Food model systems are better explained when using one gel or component; this understanding of the variation in chemical contents is needed before attempting to change their composition.

Understanding the mobility of bacteria within a food system is important when evaluating surface sanitizing treatments, especially when low penetration can be achieved. The assumption that bacteria remains in the surface is relative, because most food products are either porous or have a high water content.

Before attempting to understand the kinetics of microbial recovery a fundamental study of the factors (pH, water activity, temperature) that affect the microbial damage is needed. In this research project the conclusions were limited to the assumption that sugars restrict or optimize the conditions to recover damaged population.

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APPENDIX A

RADIATION D_{10} VALUES FOR THE MOST COMMON FOOD PATHOGENS

Table A.1

Radiation D_{10} values for *Escherichia coli* in nutritional conditions similar to the proposed fruit model system

Strain	Substrate	D_{10}	Temp. (ºC)	Irradiation Source	Reference
B/r	66.7mM Pi[1]	0.011*	2	X-ray	Hollaender et al (1951)
B/r	66.7mM Pi[1]	0.012*	2	X-ray	Hollaender et al (1951)
B/r	66.7mM Pi[1]	0.32*	2	X-ray	Hollaender et al (1951)
B/r	66.7mM Pi[1]	0.36*	2	X-ray	Hollaender et al (1951)
B/r	Saline-Pi[2]	0.09*	-	X-ray	Hollaender et al (1951)
B/r	Saline-Pi[2]	0.34*	-	X-ray	Hollaender et al (1951)
Wild	Water	-3E/5mW-s	-	UV	Butler et al (1987)
ATCC11229	Water	-3E/6.7mW-s	-	UV	Chang et al (1985)
E. coli O157 (non-verotoxin type)	Saline solution (10⁻⁷ concentration)	0.5	-20	Gamma	Niyahara and Niyahara (2002)
E. coli O157 (non-verotoxin type)	Saline solution (10⁻⁷ concentration)	0.48	-20	E-beam	Niyahara and Niyahara (2002)
E. coli O157 (non-verotoxin type)	Saline solution (10⁻⁷ concentration)	0.22	-20	Gamma	Niyahara and Niyahara (2002)

Modified from ICMSF (1996) with additions.
*Estimated from reference.
[1]mM Pi: mM Phosphate.
[2]130mM Nacl: 20mM phosphate 7.0.
mW-s: milliWatt-sec/cm^{2}.

Table A.2

Radiation D_{10} values for *Listeria monocytogenes* in nutritional conditions similar to the proposed fruit model system

Substrate	D_{10}	Temp. (ºC)	Irradiation Source	Reference
TSB	0.27	2 to 4	Cs-137	Huhtanen et al. (1989)
TSB	0.33	2 to 4	Cs-137	Huhtanen et al. (1989)
TSB	0.35	2 to 4	Cs-137	Huhtanen et al. (1989)
Phosphate Buffer	0.18	0 to 0.5	Co-60 (12.2 kGy/h)	Hashisaka et al. (1989)
Phosphate Buffer Saline	0.32 - 0.49	12	Co-60	Diaa El Din et al (1990)
TSB	0.34 to .50	-	Co-60 (0.76 kGy/h)	El Shanawy et al. (1989)
TSB	0.32 to 0.44	-	Co-60 (0.76 kGy/h)	El Shanawy et al. (1989)
TSB+ YEA	0.21 - 0.46	0 to 0.5	Co-60 (12.2 kGy/h)	Diaa El Din et al (1990)
Saline sol. (10^{-7} concentration) anaerobic.	0.5	-20	Gamma	Niyahara and Niyahara (2002)
Saline sol. (10^{-7} concentration) anaerobic.	0.48	-20	E-beam	Niyahara and Niyahara (2002)
Saline sol. (10^{-7} concentration) anaerobic.	0.22	-20	Gamma	Niyahara and Niyahara (2002)

Modified from ICMSF (1996).

TSB: Tryptic Soy Broth.

YEA: Yeast Extract Agar.

Table A.3

Radiation D_{10} values for different *Salmonella* species in nutritional conditions similar to the proposed fruit model system

Specie	Substrate/food	D_{10}	Temp. (ºC)	Reference
anatum	NB 0.3% YE	0.52	-	Epps and Idziak (1970)
blockley	NB 0.3% YE	0.48	-	Epps and Idziak (1970)
enteritidis	NB 0.3% YE	0.27	-	Epps and Idziak (1970)
give	NB 0.3% YE	0.42	-	Epps and Idziak (1970)
infantis	NB 0.3% YE	0.28	-	Epps and Idziak (1970)
manhattan	NB 0.3% YE	0.19	-	Epps and Idziak (1970)
montevideo	NB 0.3% YE	0.30	-	Epps and Idziak (1970)
pullorum	NB 0.3% YE	0.32	-	Epps and Idziak (1970)
senftenberg	NB 0.3% YE	0.25	-	Epps and Idziak (1970)
typhimurium	NB 0.3% YE	0.32	-	Epps and Idziak (1970)
Worthington	NB 0.3% YE	0.46	-	Epps and Idziak (1970)
typhimurium	BYA/ cauliflower	0.497	3	Grant and Patterson (1992)
typhimurium	BYA / cauliflower	0.518	3	Grant and Patterson (1992)
typhimurium	TSAYE / cauliflower	0.549	3	Grant and Patterson (1992)
typhimurium	TSAYE / cauliflower	0.590	3	Grant and Patterson (1992)
anatum	PB	0.116	7	Thayer et al (1990)
newport	PB	0.152	7	Thayer et al (1990)

Table A.3 Continued,

Specie	Substrate/food	D_{10}	Temp. (ºC)	Reference
enteritidis	PB	0.172	7	Thayer et al (1990)
arizonae	PB	0.184	7	Thayer et al (1990)
typhimurium	PB	0.199	7	Thayer et al (1990)
newport	BHI	0.212	7	Thayer et al (1990)
typhimurium	BHI	0.22	7	Thayer et al (1990)
arizonae	BHI	0.244	7	Thayer et al (1990)
enteritidis	BHI	0.264	7	Thayer et al (1990)
Dublin	PB	0.267	7	Thayer et al (1990)
anatum	BHI	0.288	7	Thayer et al (1990)
Dublin	BHI	0.341	7	Thayer et al (1990)
gallinarum	Buffer	0.13	RT	Ley et al (1963)
senftenberg	Buffer	0.13	RT	Ley et al (1963)
paratyphi B	Buffer	0.19	RT	Ley et al (1963)
gallinarum	Buffer	0.21	Frozen	Ley et al (1963)
typhimurium	Buffer	0.21	RT	Ley et al (1963)
seftenberg	Buffer	0.30	Frozen	Ley et al (1963)
typhimurium	Buffer	0.32	Frozen	Ley et al (1963)
gallinarum	Buffer	0.36	RT	Ley et al (1963)

Table A.3 Continued,

Specie	Substrate/food	D_{10}	Temp. (ºC)	Reference
seftenberg	Buffer	0.39	RT	Ley et al (1963)
paratyphi B	Buffer	0.49	Frozen	Ley et al (1963)
tiphymurium	Buffer	0.62	RT	Ley et al (1963)
parathyphi	Buffer	0.66	RT	Ley et al (1963)
enteritidis	Saline solution (10^{-7} concentration) anaerobic cond.	0.46 Gamma	-20	Niyahara and Niyahara (2002)
enteritidis	Saline solution (10^{-7} concentration) anaerobic cond.	0.36 E-beam	-20	Niyahara and Niyahara (2002)
enteritidis	Saline solution (10^{-7} concentration) stored at 4ºC before treatment, anaerobic cond.	0.53 Gamma	-20	Niyahara and Niyahara (2002)

Modified from ICMSF (1996).

APPENDIX B

BACTERIA STRAINS EVALUATED IN STUDIES TO FIND A SURROGATE FOR THE MOST COMMON PATHOGENS ENCOUNTERED IN FRUITS

Table B.1

Strains evaluated for antibiotic resistance to find a suitable surrogate for thermal treatments of fresh fruits

Strain	Designation	Approved
E. coli K12	ATCC 25253	Yes
E. coli K12	LMM 1010	Yes
E. coli HB 101	ATCC 33694	No
E. coli O124:NM	ATCC 43893	No
E. coli	ATCC 15490	No
E. coli 078:H7	ATCC 35401	No
E. coli	O18A, 18L:K7C B12 H7	No
L. innocua	137	Yes
L. innocua	136	No
L. innocua	227	No
L. grayi	ATCC 19120	No
L. welshimeri	ATCC 35897	No
L. grayi	ATCC 25401	No
L. welshimeri	ATCC 35967	No
L. ivanovii	ATCC 19119	No
L. innocua	ATCC 33090	No
Klebsiella pneumoniae	ATCC 9997	No
Enterobacter aerogenes	ATCC 13048	No
Enterobacter aerogenes	DSS – 1	No
Citrobacter freundii	ATCC 8090	No
Enterococcus faecalis	ATCC 19433	No
E. faecium	FAIR-E 151	Yes
E. faecium	FAIR-E 225	Yes
E. faecium	FAIR-E 160 t1	No

Modified from Peri (2003).

APPENDIX C

NUTRITIONAL CONTENT OF FRESH PRODUCE

Table C.1

Sugars content in fruits at different maturity stages

		% of Fresh Weight		
		Total Sugars	Fructose	Glucose	Sucrose
1	Apple (Glockenapfel)	9.70	4.80	0.90	4.00
2	Apricots	5.06	2.40	2.25	0.41
3	Fig	24.79	14.00	9.00	1.79
4	Kiwi (Basilicata)	11.71	5.38	5.61	0.72
5	Kiwi (Calabria)	8.58	4.73	3.85	0.00
6	Kiwi (Campania)	6.84	3.66	3.03	0.15
7	Kiwi (Piemonte)	7.78	4.00	3.78	0.00
8	Kiwi (Toscana)	8.54	4.45	4.09	0.00
9	Kiwi (Friuli)	8.80	4.58	4.22	0.00
10	Kiwi (Lazio)	11.47	5.72	5.66	0.09
11	Kiwi (Puglia)	10.08	5.10	4.98	0.00
12	Kiwi (Veneto)	8.75	4.68	4.07	0.00
13	Litchi (Groff)	12.51	5.04	5.05	2.42
14	Litchi (Shui Dong)	10.17	3.24	3.21	3.72
15	Litchi (Gui Wei)	13.40	3.78	3.48	6.14
16	Litchi (Mei Selection)	13.24	4.11	4.09	5.04
17	Mango	14.24	4.33	6.99	2.92
18	Melon (Galia)	7.50	2.00	2.00	3.50
19	Melon (Noy Yizre'el)	7.30	1.40	1.60	4.30
20	Melon (Prince)	7.11	1.99	1.93	3.19
21	Melon (Makdimond)	8.46	1.76	1.16	5.54
22	Pineapple	1.16	0.05	0.48	0.63
23	Sapodilla (Cricket Ball)	14.07	6.30	5.65	2.12
24	Sapodilla (Oblong)	15.06	4.25	7.71	3.10
25	Soursop	1.05	0.32	0.30	0.43
26	Apricots (Green Mature)		1.50	1.87	0.28
27	Apricots (Ripe)		2.31	2.25	0.40
28	Apricots (Overripe)		4.50	4.23	0.78
29	Mangoes (Green Mature)		3.60	3.34	3.94
30	Mangoes (Ripe)		4.33	6.99	2.92
31	Mangoes (Overripe)		8.91	8.17	3.10
32	Melon, Piel de Sapo (Early mmature)		1.64	1.67	0.10
33	Melon, Piel de Sapo (Inmature)		1.92	1.99	0.21
34	Melon, Piel de Sapo (Early ripening)		2.34	2.29	1.59
35	Melon, Piel de Sapo (Moderately ripe)		1.42	1.51	8.23

Table C.1 Continued,

		% of Fresh Weight			
		Total Sugars	**Fructose**	**Glucose**	**Sucrose**
36	Melon, Piel de Sapo (Ripe)		1.44	1.56	9.54
37	Melon, Rochet (Early mmature)		1.82	1.86	0.11
38	Melon, Rochet (Inmature)		2.32	2.36	0.14
39	Melon, Rochet (Early ripening)		2.19	2.24	3.06
40	Melon, Rochet (Moderately ripe)		1.13	1.36	7.01
41	Melon, Rochet (Ripe)		1.05	1.30	9.66
42	Bananas (Green)	1.00	N/A	N/A	0.70
43	Bananas (Ripe)	19.70	N/A	N/A	12.50
44	Apple	N/A	6.00	2.00	4.00
45	Banana	N/A	4.00	6.00	7.00
46	Grape	N/A	7.00	8.00	6.00
47	Orange (juice)	N/A	2.00	5.00	5.00
48	Peach	N/A	1.00	1.00	7.00
49	Strawberry	N/A	2.00	3.00	1.00
50	Tomato	N/A	2.00	2.00	0.00
	Mean	*9.93*	*3.51*	*3.42*	*2.89*
	Standard Deviation	*5.25*	*2.43*	*2.21*	*3.07*
	Max	*24.79*	*14.00*	*9.00*	*12.50*
	Minimum	*1.00*	*0.05*	*0.30*	*0.00*

Modified from Sharaf et al. (1989), Agravante, et al. (1990), Tucker (1993), Wang (1994), and Villanueva (2004).

*First 25 fruits are in the ripe stage.

Table C.2

Composition of Melons depending on maturity level

Days after anthesis (flower development)	Fructose (%)	Glucose (%)	Sucrose (%)	Total (%)
20	1.41 ± 0.10	1.39 ± 0.17	0.00 ± 0.00	2.79 ± 0.26
24	1.74 ± 0.18	1.69 ± 0.13	0.00 ± 0.00	3.43 ± 0.31
34	1.89 ± 0.19	1.62 ± 0.24	1.75 ± 0.71	5.26 ± 1.14
39	1.73 ± 0.23	1.39 ± 0.19	3.20 ± 1.32	6.32 ± 1.74
44	1.73 ± 0.14	1.14 ± 0.19	5.32 ± 1.06	8.19 ± 1.39
47	1.65 ± 0.25	0.99 ± 0.22	6.33 ± 1.05	8.97 ± 1.52

From Wang, Y. M. (1994).

APPENDIX D

PHYSICAL PROPERTIES OF GEL SYSTEMS
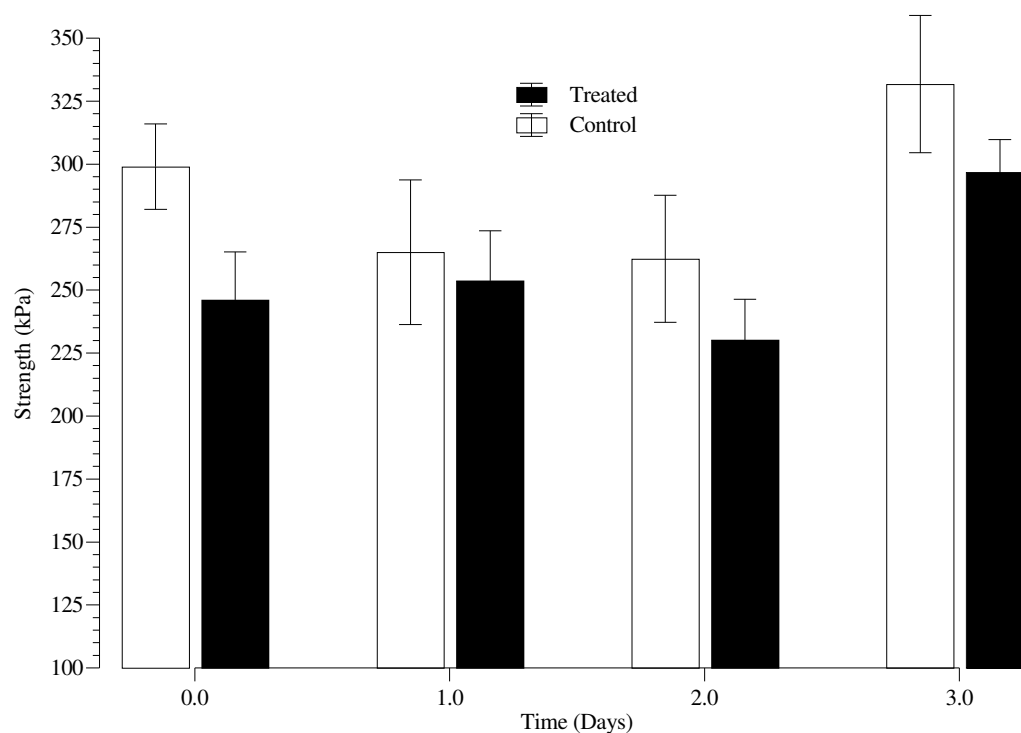
Figure D.1. Strength of gel systems without sugars (*C*) non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
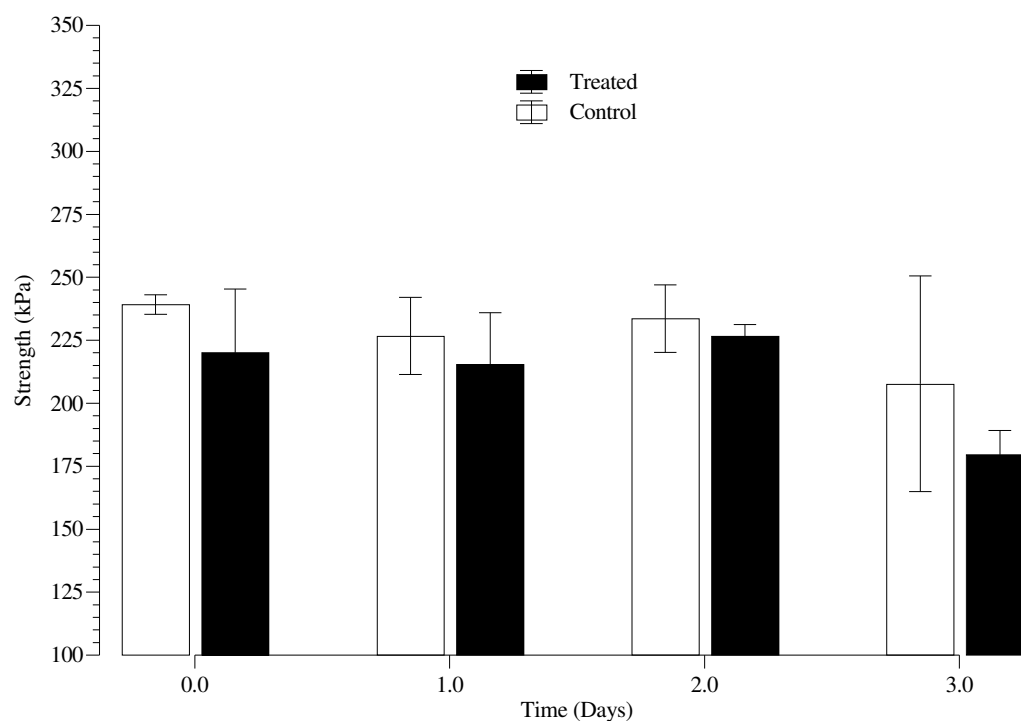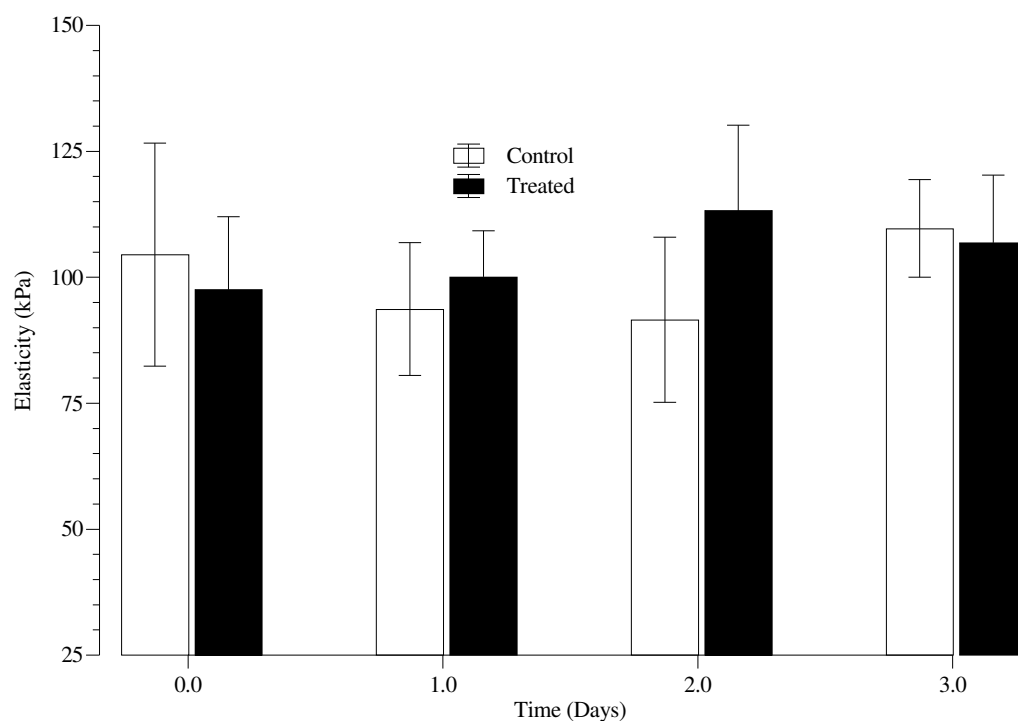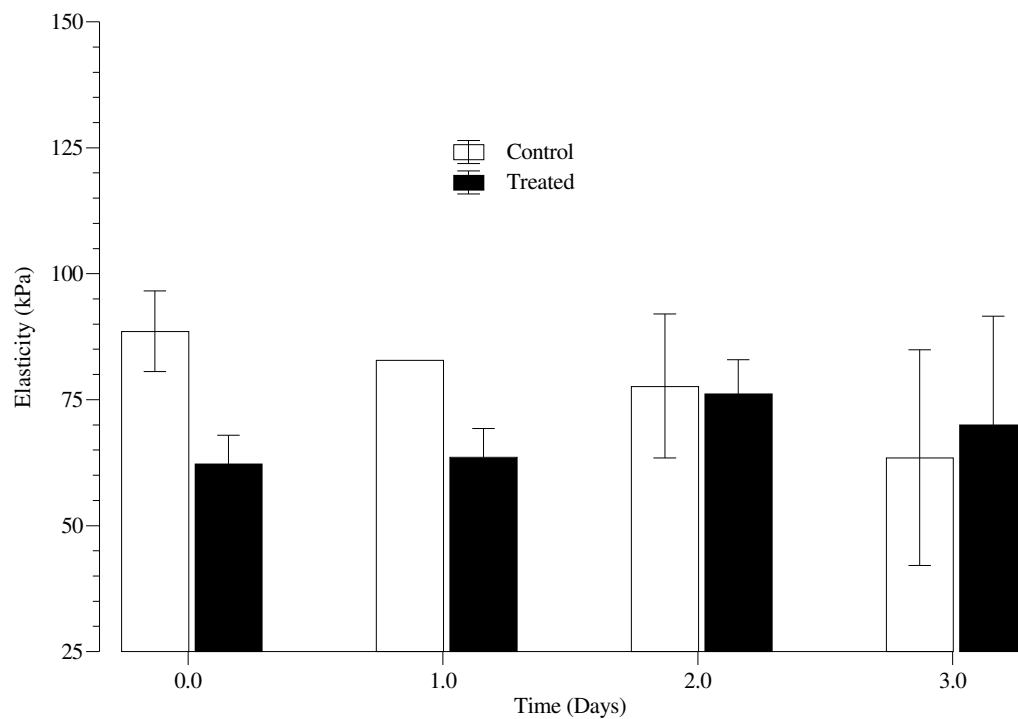
Figure D.2. Strength of Early Ripe (*ER:* 3.0% w/v, 1.5 glucose : 1.5 fructose : 0 sucrose)**)** gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
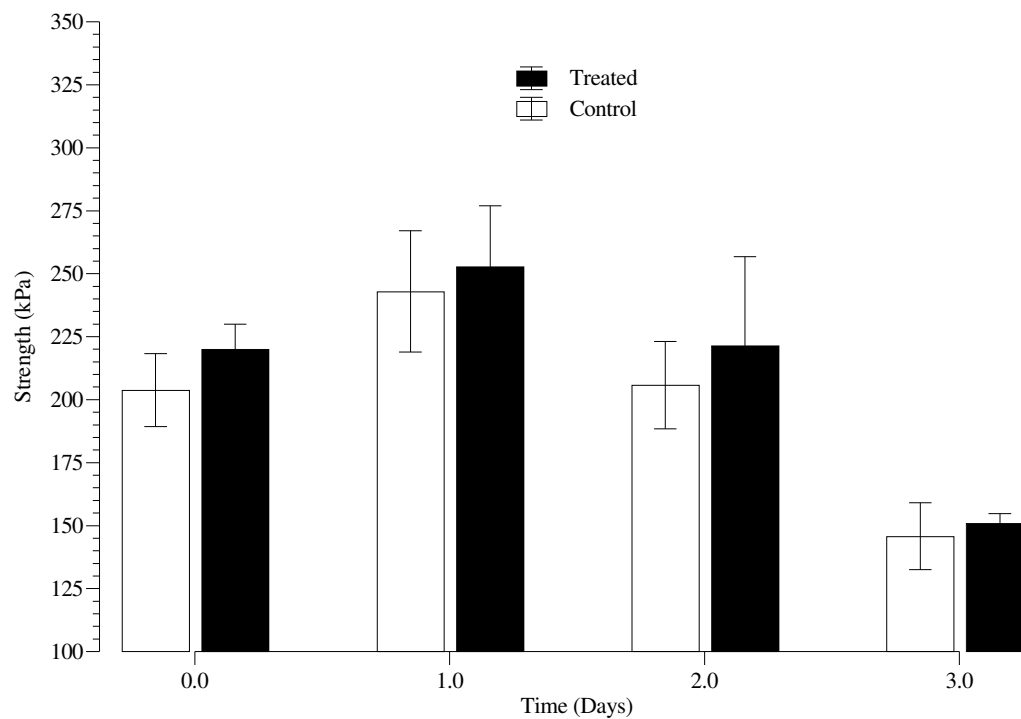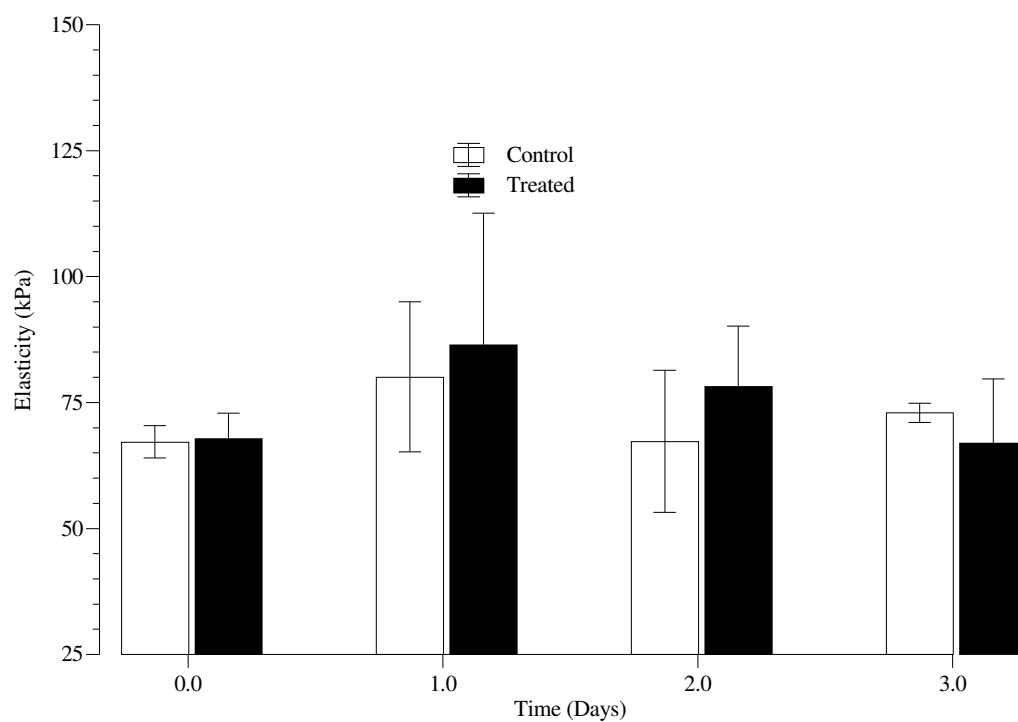
Figure D.3. Strength of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
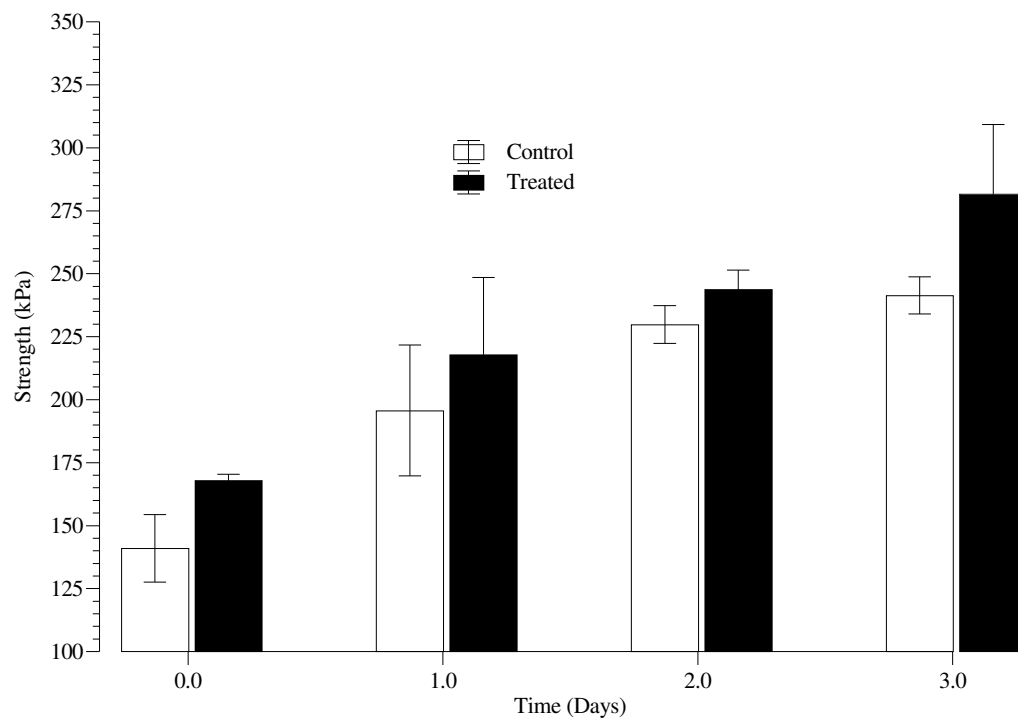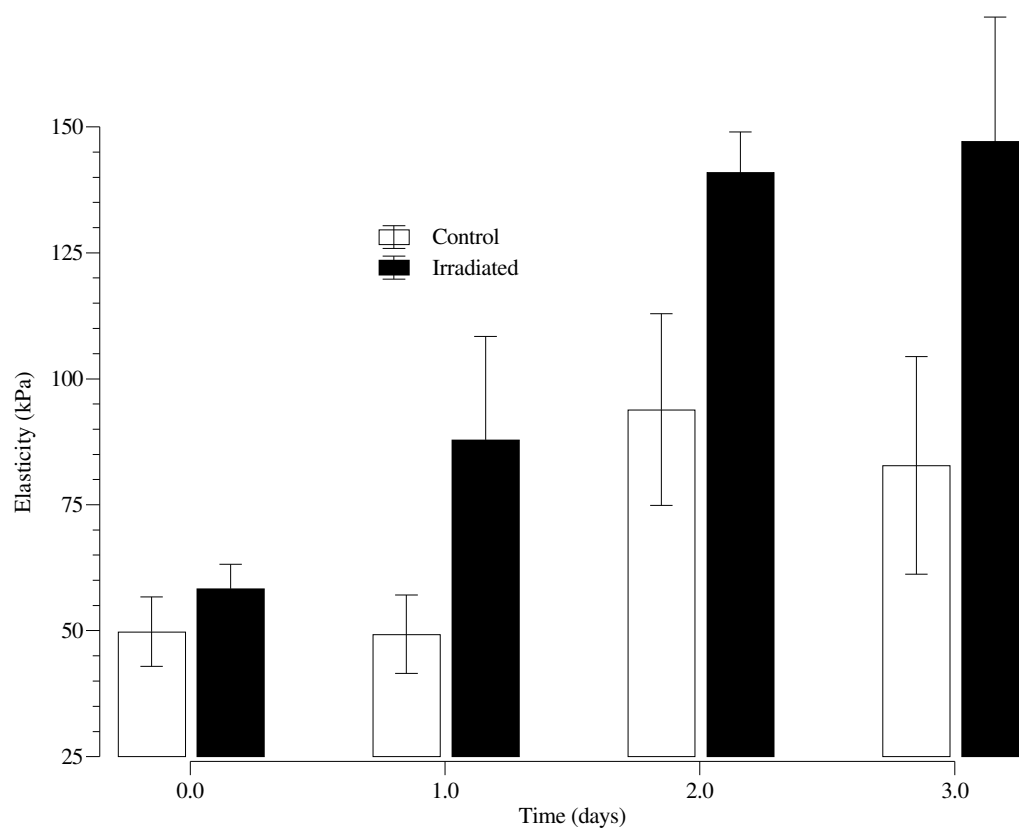
Figure D.4. Strength of Ripe (*R:* 8.0% w/v, 0.5 glucose : 0.5 fructose : 2 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
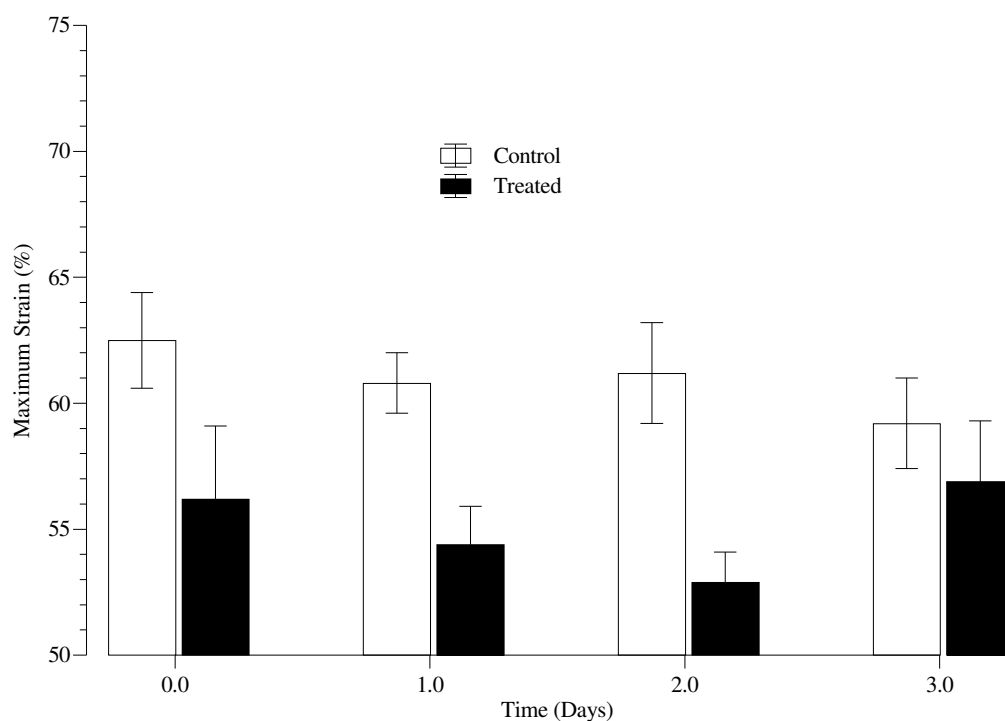
Figure D.5. Modulus of elasticity (*E*) of gel systems without sugars (*C*) non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).

Figure D.6. Modulus of elasticity (*E*) of Early Ripe (*ER:* 3.0% w/v, 1.5 glucose : 1.5 fructose : 0 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
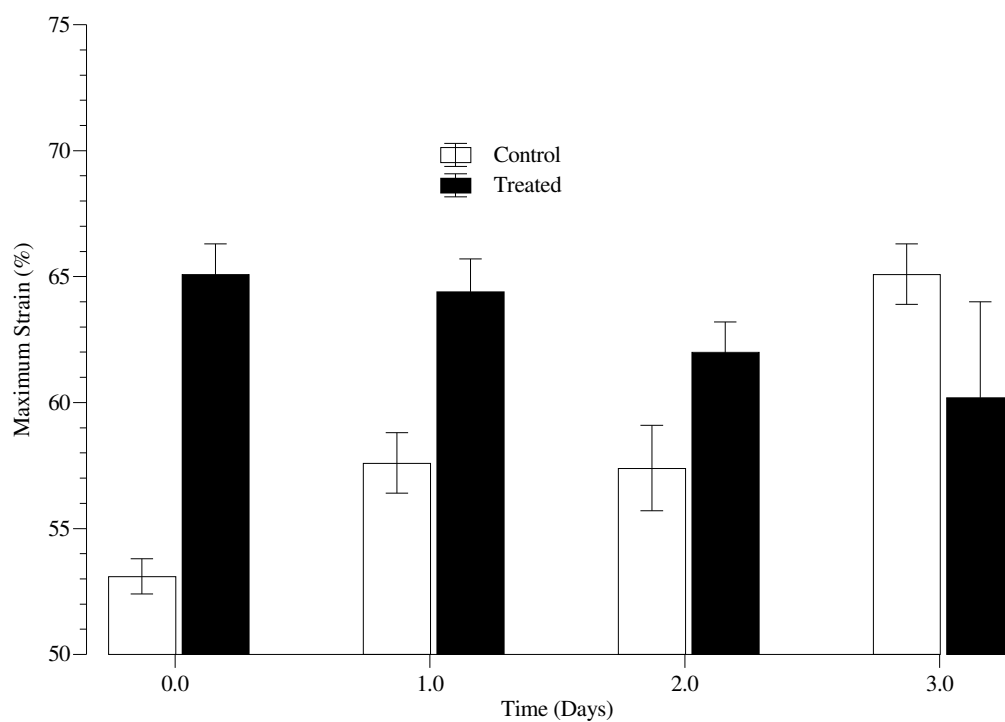
Figure D.7. Modulus of elasticity (*E*) of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
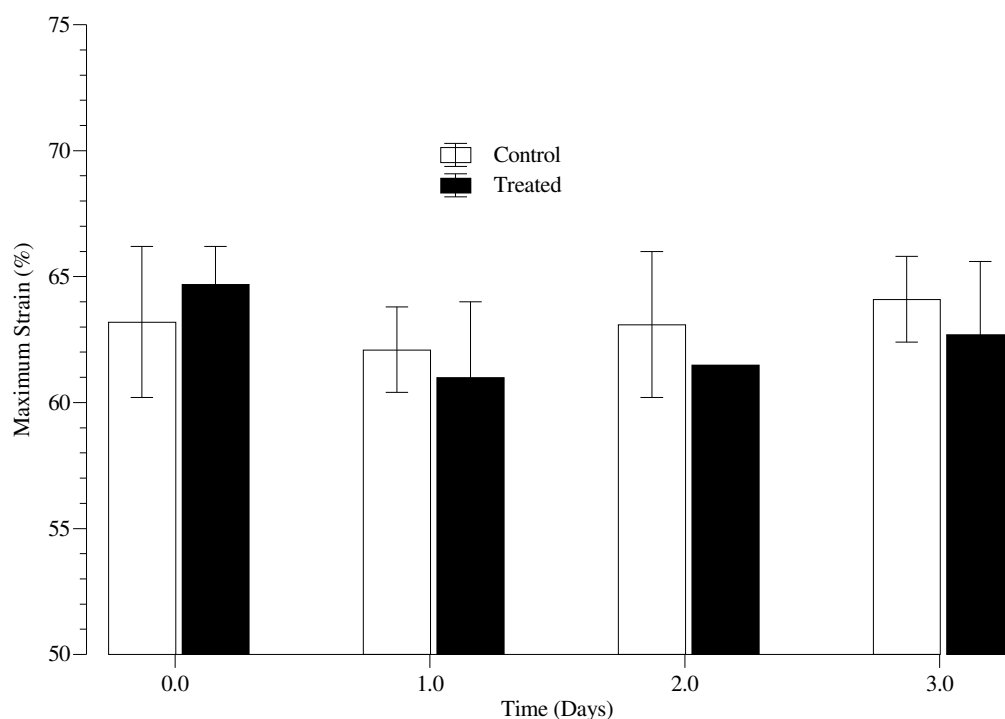
Figure D.8. Modulus of elasticity (*E*) of Ripe (*R:* 8.0% w/v, 0.5 glucose : 0.5 fructose : 2 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
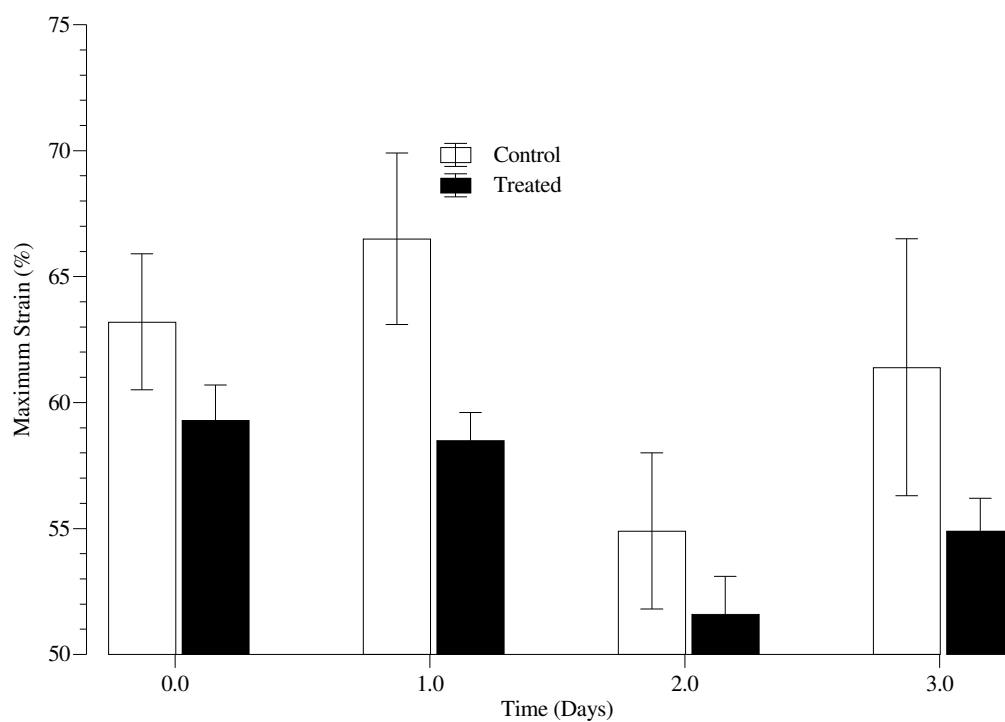
Figure D.9. Maximum Strain (γ_{max}) of gel systems without sugars (*C*) non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
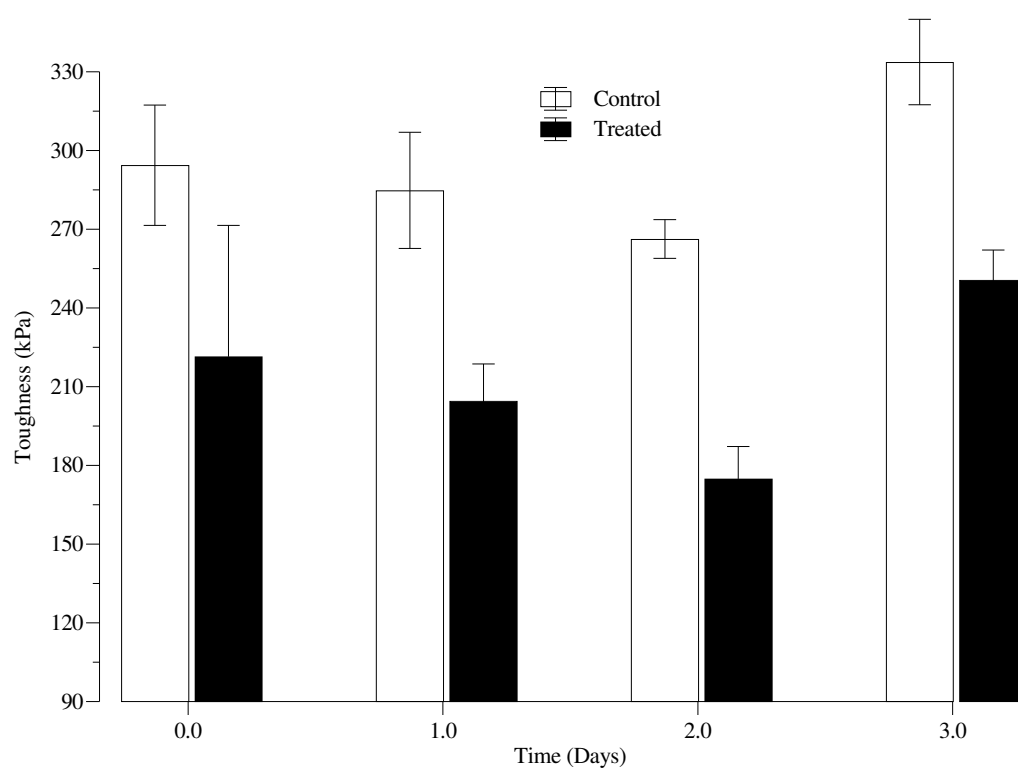
Figure D.10. Maximum Strain (γ_{max}) of Early Ripe (*ER :* 3.0% w/v, 1.5 glucose : 1.5 fructose : 0 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).

Figure D.11. Maximum Strain (γ_{max}) of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
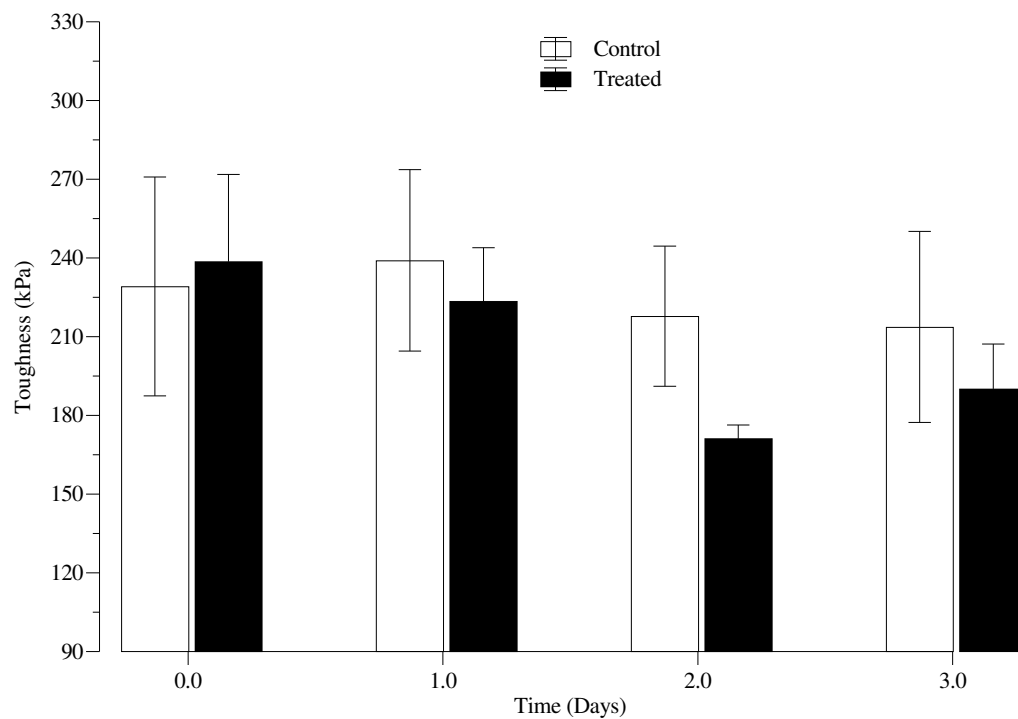
Figure D.12. Maximum Strain (γ_{max}) of Ripe (*R :* 8.0% w/v, 0.5 glucose : 0.5 fructose : 2 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
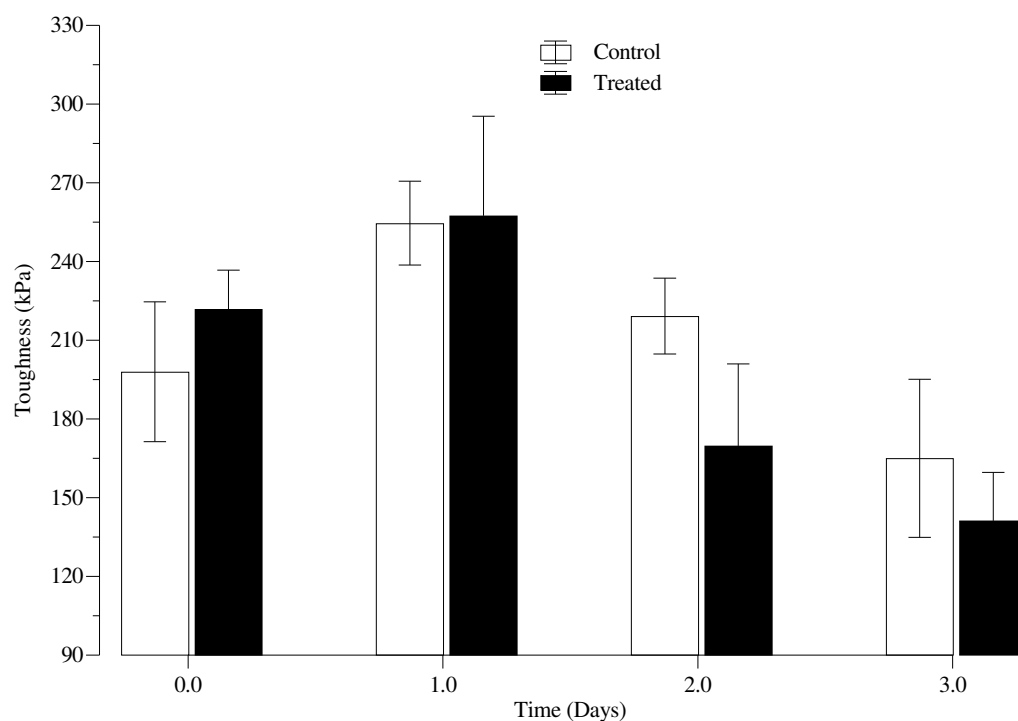
Figure D.13. Toughness of gel systems without sugars (*C*) non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
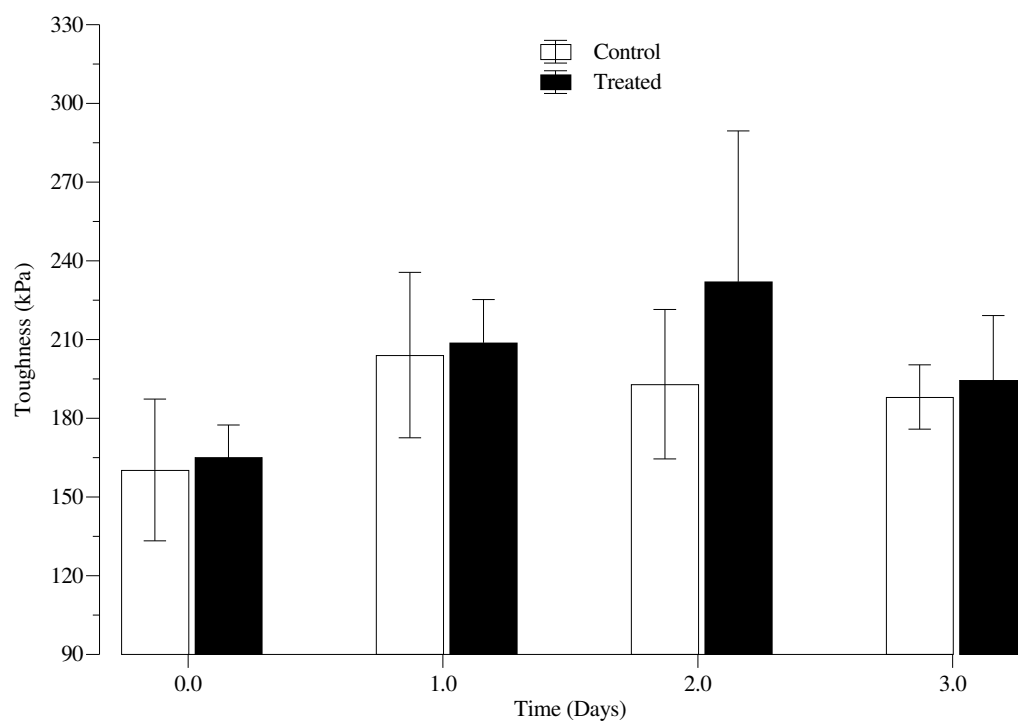
Figure D.14. Toughness of Early Ripe (*ER:* 3.0% w/v, 1.5 glucose : 1.5 fructose : 0 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
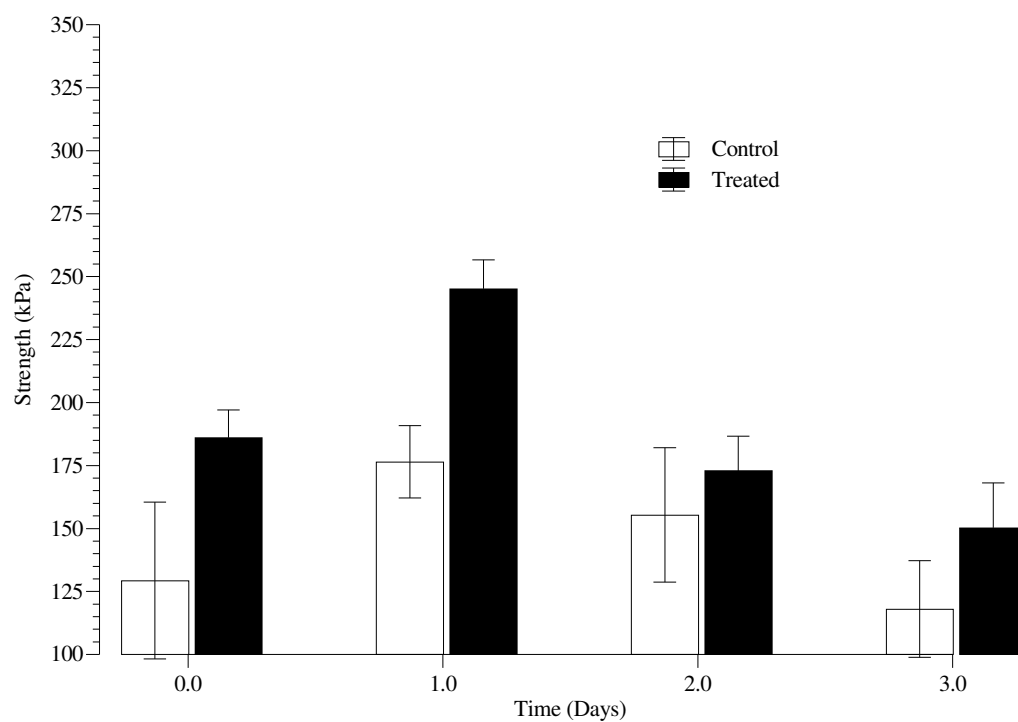
Figure D.15. Toughness of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).

Figure D.16. Toughness of Ripe (*R:* 8.0% w/v, 0.5 glucose : 0.5 fructose : 2 sucrose) gel systems non-irradiated (Control) and Irradiated stored at 4° C.

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
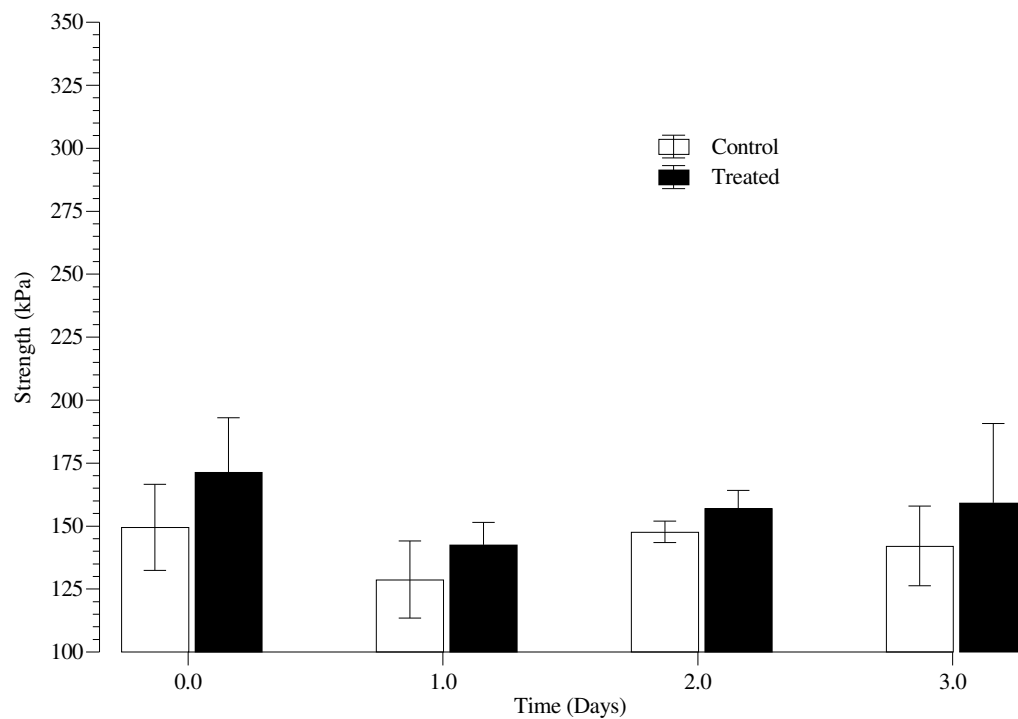
Figure D.17. Strength of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at medium temperature (10°C).

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
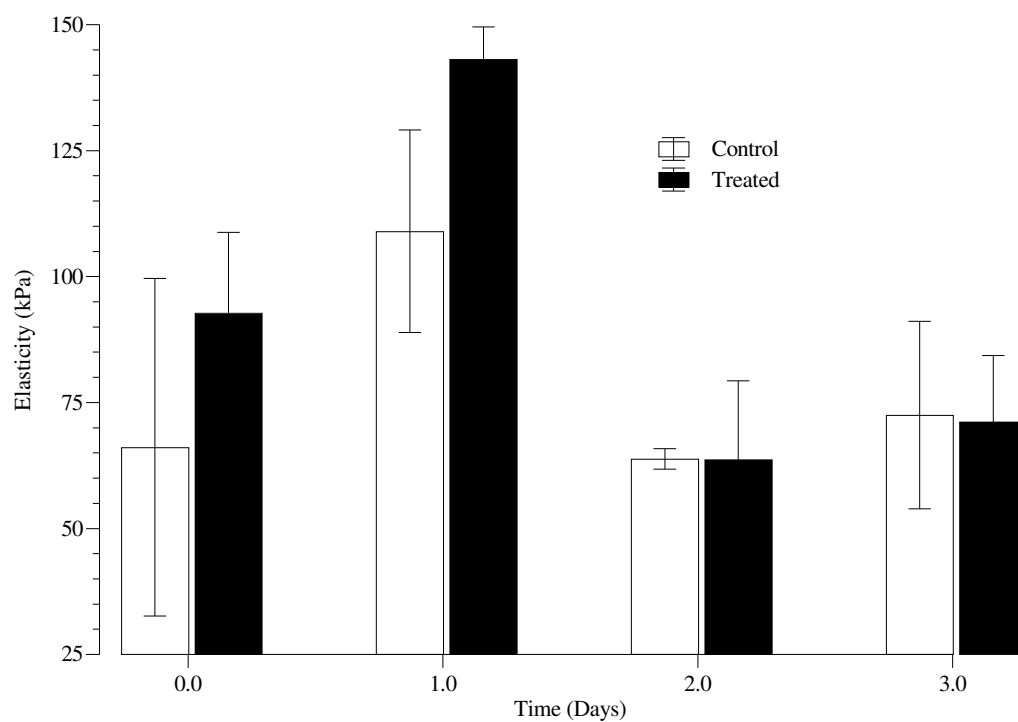
Figure D.18. Strength of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at room temperature (20°C).

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
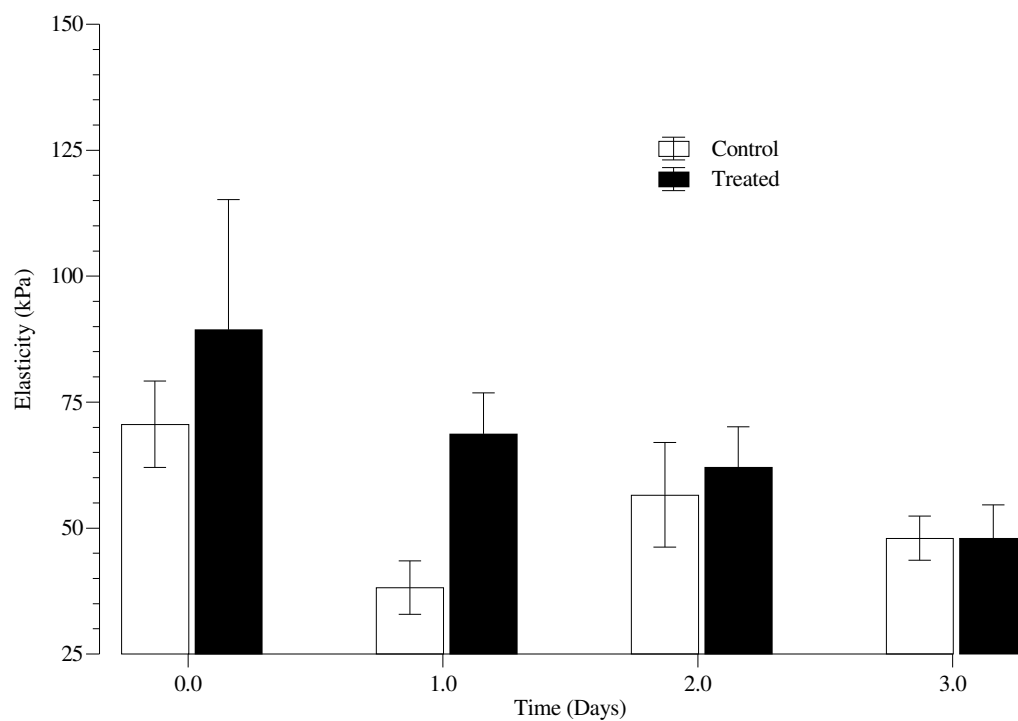
Figure D.19. Modulus of elasticity [*E*] of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at medium temperature (10°C).

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).

Figure D.20. Modulus of elasticity [*E*] of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at room temperature (20°C).

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
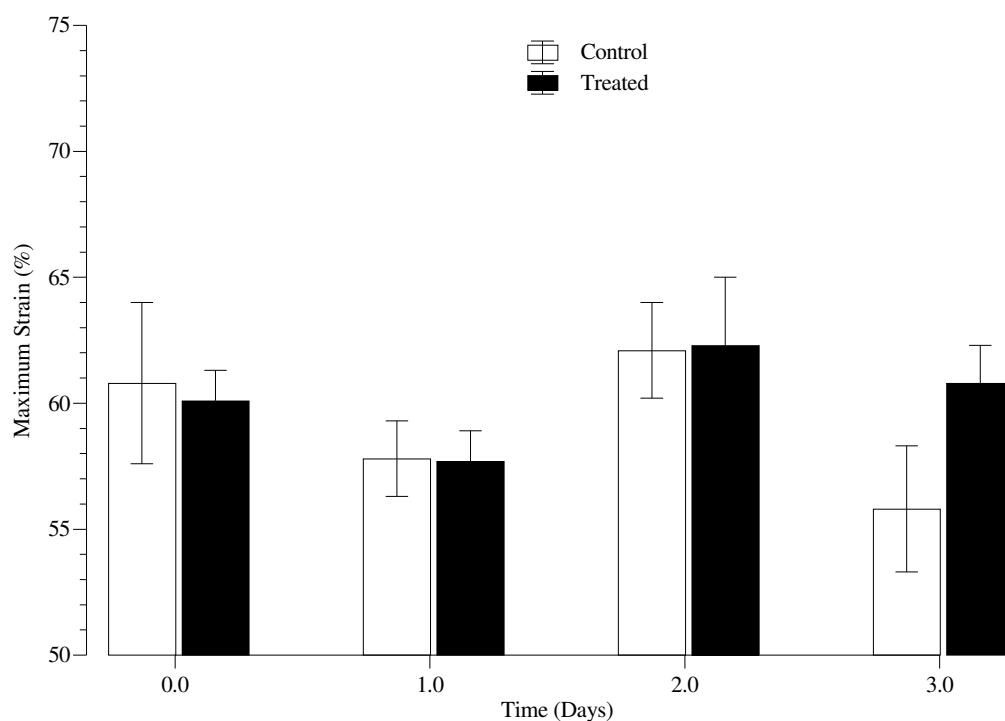
Figure D.21. Maximum Strain [γ_{max}] of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at medium temperature (10°C).

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
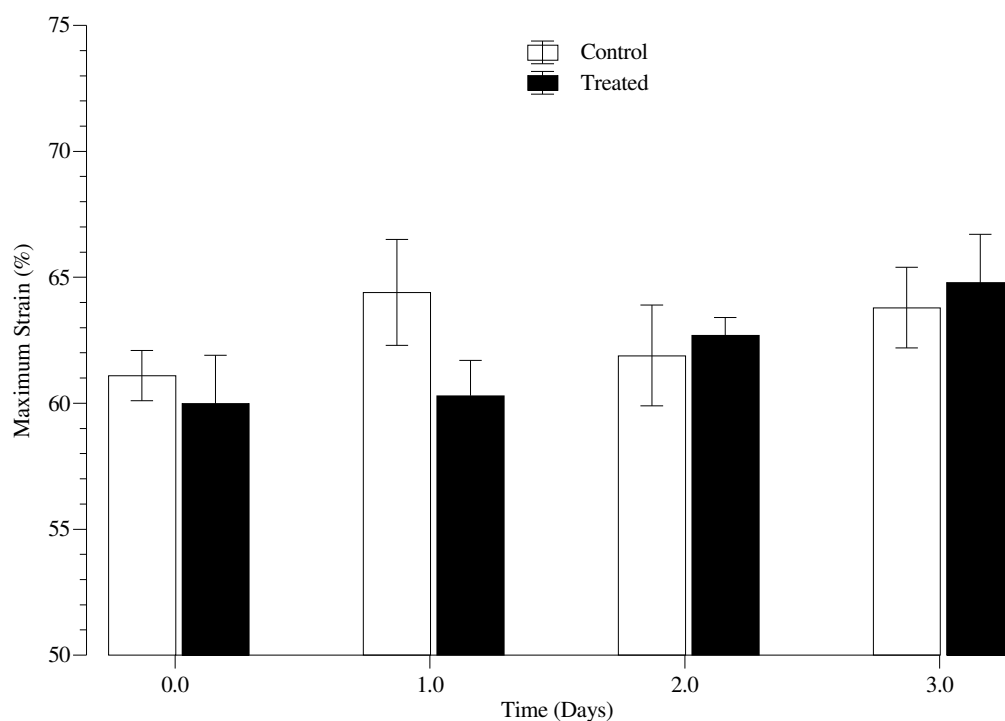
Figure D.22. Maximum Strain [γ_{max}] of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at room temperature (20°C).

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
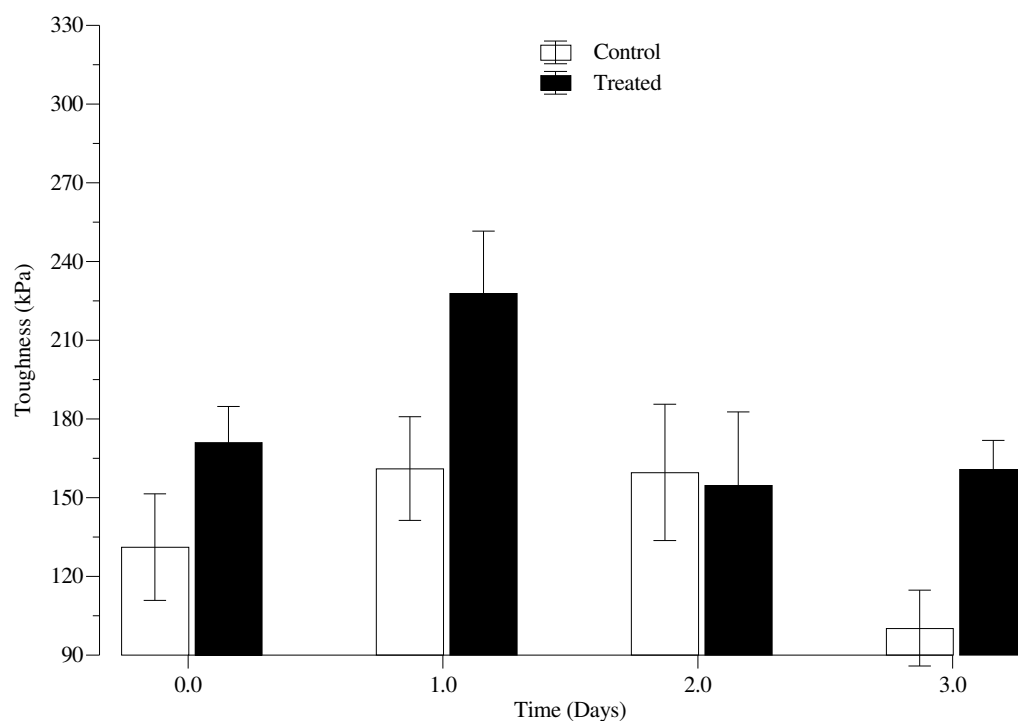
Figure D.23. Toughness of Moderately Ripe (*MR:* 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at medium temperature (10°C).

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).
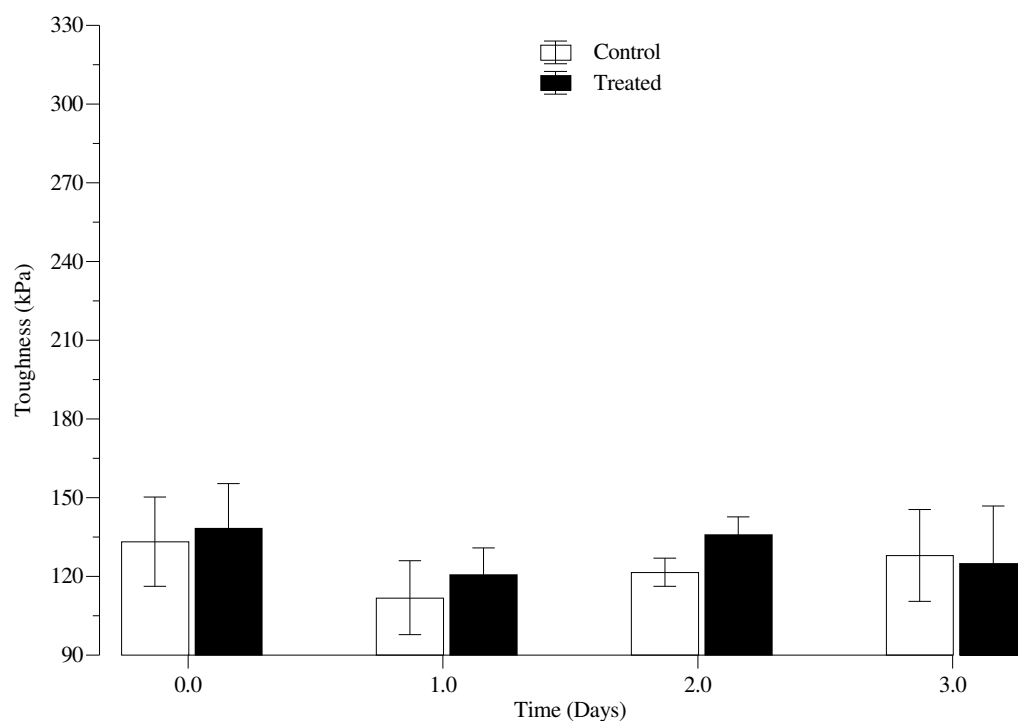
Figure D.24. Toughness of Moderately Ripe (*MR*: 5.5% w/v, 1 glucose : 1 fructose : 1 sucrose) gel systems non-irradiated (Control) and Irradiated stored at room temperature (20°C).

Samples were irradiated at 1.0 kGy using the *5C1* (5 cylinders – 1.0 cm height and 1.8 cm diameter, 22.5°, 30.5 cm from beam exit) configuration under a 2.0 MeV Van De Graaff linear accelerator (Irradiation temperature: 20°C). Texture analysis was conducted using a TA.XT2 Texture Analyzer at 0.5 mm/s and a force of 0.05 N. (n = 5).

VITA

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