UNDERSTANDING AND QUANTIFYING THE ROLE OF AQUEOUS SOLUTIONS ON THE ANTIMICROBIAL EFFECTIVENESS OF ELECTRON BEAM IRRADIATION APPLIED TO FRESH PRODUCE

A Dissertation

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ABSTRACT

The main aim of this study was to enhance the safety if fresh produce in general, and grape tomatoes in particular, by the combined treatments of electron beam (e-beam) irradiation and hydrogen peroxide (H_2O_2) aqueous solution. For the achieving this aim, this study was divided into three steps. At first and second steps, the effect of culture media used for the preparation of inoculum and water quality parameters, respectively, on the effectiveness of e-beam irradiation to inactivate *Salmonella* Typhimurium were evaluated. At final step, the decontamination ability of the combined treatment of e-beam and H_2O_2 for the inactivation of *Salmonella* spp. in whole grape tomatoes was evaluated.

In the first step, the radiation sensitivity (D_{10} value) of the pathogen in deionized (DI) water decreased ($P < 0.05$) by 19.73% and 26.53% with the addition of PW and PBS, respectively, into DI water. Accordingly, the addition of 10 mM and 50 mM phosphate buffer into DI water decreased ($P < 0.05$) the radiation sensitivity of *S.* Typhimurium in DI water by 6.12% and 32.65%, respectively. Similarly, the calculated D_{10} value for this bacterium in 10 mM PB increased ($P < 0.05$) by 19.23% when 150 mM NaCI was added into 10 mM PB. However, the addition of 1.0 mM PB into water did not affect ($P > 0.05$) the D_{10} value calculated for *S.* Typhimurium in DI water.

In addition, the presence of hydroxyl radical scavengers, ethanol and polyethylene glycol (PEG), provided a protection to *S.* Typhimurium cells. The radiation sensitivity of the pathogen in buffer solution decreased ($P < 0.05$) by 65.51%, 162.07%,

and 250.34% when the concentration of membrane-permeable ethanol in the solution was modified as 78.9 mM, 394.5mM, and 1578 mM, respectively. Likewise, although the addition of 0.125 mM and 1.875 mM PEG into buffer solution decreased ($P < 0.05$) the radiation sensitivity of S. Typhimurium in buffer solution by 29.66% and 43.45%, respectively, 0.0125 mM PEG did not influence ($P > 0.05$) the D_{10} value calculate for the bacterium in buffer solution.

In the second step, the pH ranged from 5.5 to 8.5 and alkalinity (≤ 500mg/l) did not affect ($P > 0.05$) the radiation sensitivity of S. Typhimurium in buffer solution. Nevertheless, the radiation sensitivity of *S.* Typhimurium in buffer solution increased ($P < 0.05$) when fulvic acid (100 mg/l ≤) and nitrate (≥ 100 mg/l) was added into buffer solution as organic and inorganic substances, respectively. In addition, the radiation sensitivity of *S.* Typhimurium in buffer solution increased ($P < 0.05$) regardless of H_2O_2 concentration.

In the third step, it is found that this integrated treatment was effective to inactivate *Salmonella* spp. in grape tomatoes. The calculated D_{10} value for *Salmonella* spp. on grape tomatoes was 0.25 kGy. In addition, this system improved uniform dose distribution throughout tomatoes. Furthermore, the e-beam dose up to 1.25 kGy did not affect the quality of tomatoes.

Ultimately, the present study demonstrated that the combined treatments of e-beam and H_2O_2 aqueous solution could be a promising alternative to conventional processes and should enhance the safety of fresh produce.

DEDICATION

To my parents, Mehmet and Medine OMAC

To my siblings, Zeki, Hayriye, Ramazan, Fatma, Emine, and Yasin OMAC

To my nieces, Aysegul, Betul, and Simay OMAC, Tugba, DONMEZ

To my nephews, Taha, Utku, Ali Haydar, and Bartu OMAC, Mehmet, Enes, and Ibrahim KUNUK, Abdulsamet, Kerim, and Burak TETIK, Ensar DONMEZ

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CHAPTER I

INTRODUCTION

The production and consumption of fresh and minimally processed fruits and vegetables has increased steadily in the United States (U.S.) because they are crucial natural sources of essential nutrients necessary to maintain a healthy diet. The total per capita availability of fresh fruits and vegetables averaged 116 and 144.5 pounds in 2016, up 3.20 and 2.0 % from 2015, respectively, (Minor and Bond, 2017; USDA, 2018). More specifically, the production of tomatoes in 2017 was 2,845.4 million pounds which was 1.0 % higher than that for 2016 (Parr et al., 2018). Although their fresh and nutritional status is appreciated, the occurrence of foodborne illnesses outbreaks linked to these products is continually raising with many incidents associated with pathogenic bacteria such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* (Painter et al., 2013; Gil et al., 2015), viruses (norovirus and rotavirus) (Predmore et al., 2015; Bosch et al, 2016), and parasites (*Toxoplasma gondii*) (Hohweyer et al. 2016; Meireles et al., 2016). Between 1973 and 2012, approximately 606 leafy vegetable-associated outbreaks, with 20,003 associated illnesses, 1030 hospitalizations and 19 deaths were reported (Herman et al., 2015). In addition, 15 multistate salmonellosis outbreaks attributed to the consumption of raw tomatoes resulting in 1959 illnesses, 384 hospitalizations, and 3 deaths were reported during 1973-2010 in the U.S. (Bennett et al., 2015). These outbreaks have received increased attention from fresh produce growers,

processors, and distributors regarding the safety of fresh produce because of the tremendous economic impact of outbreaks and recalls to the fresh produce supply chain.

During 2013-2015, the pathogen-food category pairs responsible for the most illnesses in outbreaks with a single confirmed etiologic agent were *Salmonella* in fresh produce in the USA (CDC, 2015, 2016a-b). The Centers for Disease Control and Prevention (CDC) also estimated that *Salmonella* caused approximately 1.2 million illnesses and 450 deaths in the United States every year (CDC, 2016c; USFDA, 2016). A more recent study pointed out that the number of *Salmonella* outbreaks in the USA has increased continuously since 2004 and *S*. Typhimurium and *S*. Newport were most included in these outbreaks, both linked primarily to the consumption of tomatoes (Callejon et al., 2015).

Fresh produce may be contaminated by foodborne pathogens such as *Salmonella* through the water, air, soil, insect vectors, and equipment (Meireles et al., 2016) and food safety intervention strategies are needed to enhance their microbial safety and to increase their shelf life. These two goals should be accomplished without negatively affecting the quality of the fresh produce. To date, the efficiency of chemical (chlorine, hydrogen peroxide, organic acids, etc.), physical (irradiation, filtration, ultrasounds, etc.), and biological (bacteriocins, bacteriophages, enzymes, and phytochemicals) methods to ensure the microbial safety of fresh produce has been reported in various reviews (Gil et al., 2009; Banach et al., 2015; Meireles et al., 2016; Banach et al., 2017). However, an effective strategy to achieve a 5-log pathogen population reduction as recommended by the United States Food and Drug Administration (USFDA) and the

International Commission on Microbiological Specifications for Food (ICMSF) is still needed (Mahmoud, 2010; Mukhopadhyay et al., 2013; Doona et al., 2015).

A range of sanitizers is allowed for use in the post-harvest washing of fresh produce to reduce microbial contamination, helping in the prevention of foodborne illness (Ramos et al., 2013). However, the effectiveness of sanitizers is affected by several factors, such as initial concentration of bacteria colonizing on the surface of produce, treatment type, the surface to be treated, the type of sanitizers, contact time, temperature of exposure to the sanitizer, pH, and water properties (Goodburn and Wallace, 2013). Moreover, the ability of these sanitizers to remove naturally present microorganisms from fresh produce is limited (0.5-3.0-log reduction) (Luo et al., 2012; Banach et al. 2015). In addition, the ability of pathogens to internalize, such as *E. coli* O157:H7 and *Salmonella* spp., is of great concern for produce safety because chemical sanitizers used at the post-harvest stage cannot reach enteric pathogens in the plant tissue (Meireles et al., 2016). Furthermore, the formation of biofilms on fresh produce can protect pathogens against antimicrobial biocides, disinfectants, and sanitizers (Almasoud et al., 2015).

Another challenge for the fresh produce industry is to maintain the water quality during washing because the concentration of sanitizer used to avoid cross contamination would be lower compared to the concentration needed for microbial inactivation in the fresh produce (Banach et al., 2015). Therefore, a water disinfection step should be added to maintain the water quality throughout processing and minimize the potential cross

contamination during washing (Gil et al., 2009). In addition, the presence of adventitious organic matter may reduce the sanitizer effectiveness (Meireles et al., 2016).

Several physical sanitizing methods such as ultraviolet-C (UV-C), ultrasound, and irradiation have been used to reduce or eliminate pathogenic microorganisms in fresh produce. The advantages of UV-C for decontaminating water, fruits, and root vegetables has been established (Artes et al., 2009; Fan et al., 2017; Huang et al., 2018a). Mukhopadhyay et al. (2014) found that UV-C doses of 0.60 -6.0 kJ/m^2 resulted in 2.3-3.5 log CFU per tomato reduction of *E. coli* O157:H7 compared to 2.15-3.1 log CFU per tomato reduction for *Salmonella* spp. on the surface of tomato. However, this treatment causes product heating and cannot penetrate into the deep interior of the produce (Guo et al., 2017). Hence, ultrasound treatment is only suitable for surface decontamination of fresh produce and exhibits less than 1.0 log reductions in the number of microorganisms on fresh produce (Yoon and Lee, 2017).

Food irradiation processing technology has significant value in terms of ensuring the safety, quality, and phytosanitary standards of fresh produce (Gomes et al., 2008; Pillai and Shayanfar, 2018; Joshi et al., 2018). This technology needs less processing time and energy compared to the other non-thermal technologies such as ultrasound and cold plasma (Ramos et al., 2013; Li and Farid, 2016). Several studies have demonstrated the high effectiveness of irradiation to eliminate foodborne pathogens on fresh produce (Gomes et al., 2009, Mahmoud, 2010; Chimbombi et al., 2011; Predmore et al., 2015). Gomes et al. (2009) determined that the electron beam irradiation up to 1.0 kGy resulted in 3-4 log reduction of internalized *E. coli* on the lettuce leaves. Shim et al. (2012)

pointed out that the gamma irradiation at 1 kGy reduced the concentration of *S.* Typhimurium on lettuce leaves by 3 log CFU per leaf. On the other hand, FDA only approves the use of a maximum level of 1.0 kGy to decontaminate fresh produce.

Irradiation affects target microorganisms by damaging their DNA, breaking down cell membrane, and interrupting enzymic pathways (Tahergorabi et al. 2012; Li and Farid, 2016). A study determined that when an aqueous DNA solution including 500 mg dm^{-3} DNA was gamma irradiated, ~99.5% of the energy of the irradiation was absorbed by the water and only ~0.5% by DNA (von Sonntag, 2006). Therefore, the major effect of irradiation is to produce short-lived and transient radicals, such as hydroxyl radical, the hydrogen atom, and hydrated electron, which lead to cell lysis (Li and Farid, 2016). Kim and Thayer (1995) suggests that most radiation-induced cell lethality in aqueous solution was related to the cooperative effects of extracellular hydroxyl radicals and oxygen on the surfaces as the radiation dose increased. In addition, there are some interfering factors including organic and inorganic substances in irradiation efficacy on water disinfection due to their reactions with hydroxyl radicals formed during irradiation (Wojnarovits et al., 2018). Hence, irradiation should be used in combination with a chemical method such as hydrogen peroxide (H_2O_2) to reduce the applied doses to achieve 5-log reduction without affecting food quality (Doona et a., 2015). H_2O_2 is a powerful oxidizer and hydroxyl radicals can be produced via H_2O_2 in aqueous solution using irradiation without increasing the radiation dose (Stefan, 2018).

The main goal of this study was to validate a strategy to increase the killing effectiveness of electron beam irradiation against *Salmonella enterica* spp. in fresh

produce. *Salmonella enterica* spp. and tomatoes were chosen because all outbreaks associated with raw tomatoes were caused by *Salmonella enterica* spp. (Bennett et al., 2015). This goal was achieved by carrying out the following specific objectives:

(1) Quantifying the effect of irradiation – in water alone or in combination with peptone water, phosphate buffered saline, phosphate buffer, and the available hydroxyl radical scavengers – on the efficacy of electron beam irradiation to inactivate *S.* Typhimurium ATCC 13311.

(2) Establishing whether water quality parameters affect the inactivation efficacy of *S.* Typhimurium ATCC 13311 under electron beam irradiation.

(3) Quantifying the effect of hydrogen peroxide (H_2O_2) on the efficacy of electron beam irradiation to inactivate *S.* Typhimurium ATCC 13311.

(4) Proposing a hurdle decontamination process - using the combination of chemical (H_2O_2) and physical (electron beam irradiation) methods - in a commercial electron beam irradiation facility to inactivate *Salmonella* spp. on grape tomatoes.

CHAPTER II

LITERATURE REVIEW

2.1 Health benefits of fruit and vegetables

The consumption of fresh fruits and vegetables has long been of interest due to their have content of carotenoids, flavonoids, vitamins, minerals, and antioxidants (Wang et al., 2014; Avalos-Llano et al., 2018; Honrath et al., 2018). These food items have been shown to reduce the risk of diseases such as cardiovascular conditions, certain cancers, Type II diabetes and obesity (CDC, 2011; Mytton et al., 2014; Rekhy and McConchie, 2014; Hosseini et al., 2017; Sharma et al., 2017).

The World Health Organization (WHO) reported that approximately 16.0 million (1.0 %) disability adjusted life years and 1.7 million (2.8 %) of deaths worldwide are associated with low consumption of fruits and vegetables (WHO, 2003). Furthermore, the Food and Agriculture Organization (FAO)/ WHO report (2004) suggests a minimum of 400 g of fruits and vegetables per day, excluding potatoes and other starchy tubers, to prevent chronic diseases such as heart disease, cancer, diabetes, and obesity, as well as to prevent and alleviate several micronutrient deficiencies. More recently, Bes-Rastrollo et al. (2006) found that there was a significant inverse association between total fruit and vegetable consumption and weight gain due to a high intake of total fiber. Similarly, Reiss et al. (2012) reported that if the consumption of fruits and vegetables by one-half of the U.S. population increased to one serving each day an estimated 20,000 cancer cases could be prevented each year. Another study concluded that fruit and vegetable

consumption improved the psychological well-being of the population and reduced the negative impact of mental health problems (Rooney et al., 2013). Based on all these facts, the United States Centers for Disease Control and Prevention (CDC) recommends that depending on their age and sex, adults should eat at least 1.5-2 cup equivalents of fruit and 2-3 cups of vegetables daily (Moore and Thompson, 2015).

2.2 Fresh produce and associated foodborne illness outbreaks

The market of fresh produce, one of the major growing sectors in the food industry, has increased drastically in recent years due to an altered lifestyle characterized by less time for planning and preparing convenient meals and a wide variety of particularly ready-to-eat or minimally processed fruits and vegetables (Patrignani et al., 2015; Pinela and Ferreira, 2017). In fact, the Produce for Better Health Foundation (2015) reported that fruit, excluding juice, and fresh vegetables consumption are expected to grow by 9% and 8%, respectively, over the next 5 years.

The U.S. market of fresh and minimally processed fruits and vegetables consists of 23% fresh fruits, 29% fresh vegetables, and 48% minimally processed fruits and vegetables (Patrignani et al., 2015). Availability of fresh fruits and vegetables per capita availability also increased by 34.2% and 20.9%, respectively, over the past 40 years (USDA, 2018a).

Foodborne illness outbreak is defined as a case in which two or more persons with a similar disease resulting from ingestion of common food in the U.S. (CDC, 2018a). The National Outbreak Reporting System (NORS), web-based platform, has been used by local, state, and territorial health departments in the U.S. for providing

epidemiological data to CDC from their all waterborne and foodborne diseases outbreak investigations (CDC, 2018b). These surveillance data have provided great insight regarding foodborne diseases and outbreaks, such as the identification of new and emerging foodborne agents, specific agent-food pairs, and the public health importance and effects of specific agents (Brown et al., 2017; CDC, 2018b)

The prevalence of reported outbreaks linked to consumption of fresh fruits and vegetables, such as tomato, lettuce, apple, carrot, and spinach, has increased in recent years (Yoon and Lee, 2017). Every year, approximately 48 million foodborne illnesses occur in the United States, resulting in about 128,000 hospitalizations and 3,000 deaths, either from 31 known pathogens or by unspecified agents (Doyle et al., 2015; Brown et al., 2017). Additionally, these 31 known pathogens caused 9.4 million episodes of foodborne illnesses, resulting in approximately 55,961 hospitalizations and 1,351 deaths each year (Scallan et al., 2011; Nguyen et al., 2015).

Based on data published by the CDC, 902 foodborne disease outbreaks were reported in 2015 (CDC, 2017). These outbreaks involved 15,202 reported cases of illness, 950 hospitalizations, 15 deaths, and 20 food products recalls. Within these outbreaks, many were associated with fresh and minimally processed produce (44%) and the most outbreak-associated diseases were from seeded vegetables, such as tomatoes or cucumbers, (26%) than to any other single commodity. Within the reported instances of foodborne illnesses, bacteria caused the most outbreaks (238 outbreaks, 54%), followed by viruses (168 outbreaks, 38%), chemicals (33 outbreaks, 7%), and parasites (4 outbreaks, 1%). Among outbreaks with known etiology, *Salmonella* was the most

common cause of hospitalizations related to foodborne disease outbreaks (62%), followed by the Shiga toxin-producing *Escherichia coli* (STEC) causing 12% of the reported, outbreak-associated hospitalizations (CDC, 2017). In addition, in a review of outbreaks associated with imported produce into the U.S. with 1996-2014, Gould et al. (2017) found that these outbreaks were mostly linked to Salmonella (77%), including fruits (35%), seeded vegetables (25%), sprouts (15%), nuts and seeds (12.5%), spices (10%), and herbs (2.5%).

The economic impact of produce-associated outbreaks is considerable regarding the medical costs and lost income of patients, and the cost of damage control (disposal of unmarketable products, product recalls, cleanups, and retrofitting), and lost production time incurred by the affected produce packer/processor (Sapers and Doyle, 2014). Furthermore, the outbreak history can damage an entire segment of the produce industry or a production area, yielding to increased costs for compliance with government-mandated adjustments in production and processing practices and in decreased sales of products nationwide (Sapers and Doyle, 2014). Some studies estimated that infections with 14 of the 31 major foodborne pathogens caused $14.0 billion in cost of illness (with the range between $4.4 billion to $33.0 billion) and a loss of 61,000 quality-adjusted life years (QALYs) (with the range between 19,000 to 145,000 QALYs) per year in the U.S. (Hoffmann et al., 2012; Robertson et al., 2016). Approximately 90% of this loss is induced by five pathogens: nontyphoidal *Salmonella* enterica ($3.3 billion; 17,000QALYs), *Campylobacter* spp. ($1.7 billion; 13,300QALYs), *Listeria monocytogenes* ($2.6 billion; 9,400QALYs), *Toxoplasma gondii* ($3.0 billion;

11,000QALYs), and norovirus ($2.0 billion; 5,000 QALYs) (Hoffman et al., 2012). In addition, Bartsch et al. (2018) estimated that the cost of a single foodborne disease outbreak ranged from $3,968 to $1.9 million for a fast-food restaurant, $6,330 to $2.1 million for a fast-casual restaurant, $8,030 to $2.2 million for a casual-dining restaurant, and $8,273 to $2.6 million for a fine-dining restaurant.

2.3 Salmonella

Salmonella spp. are gram negative, facultatively anaerobic, non-spore forming, rod-shaped bacteria belonging to the family Enterobacteriaceae, and alike from *E. coli* under the microscope or on ordinary nutrient media (Jay et al., 2005). There are more than 2,579 serotypes of *Salmonella* and many of them are not considered human pathogens (Andino and Hanning, 2015). The optimum growth temperature and pH values of *Salmonella* are 35-37 °C and 6.6-8.2, respectively. Some strains of *Salmonella* can grow in foods stored at temperature between 2 °C to 4 °C and some of them can grow at elevated temperatures of up to 54 °C (Li et al., 2013). This microorganism has ability to proliferate at pH values varied from 3.99 to 9.5 (Jay et al., 2005).

Salmonella is classified into two different species, *Salmonella bongori* and *Salmonella enterica* having six subspecies including *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), and *S. enterica* subsp. *indica* (VI) (Jay et al., 2005). Almost 60% of all serotypes of Salmonella belong to the group I which is usually found in the intestinal tract of humans and other warm-blooded animals (Li et al., 2013). Group II and III are mostly associated with col-blooded animals and

group IV and VI are environmental isolates which are rarely human pathogens (Li et al., 2013). *Salmonella* serotypes are identified depending on somatic (surface) and flagellar antigens as characterized by the Kauffman-White typing scheme (Jay et al., 2005). *S. enterica* serovar Typhimurium is one of the most common *S. enterica* serovars causing *Salmonella* diseases outbreaks in the U.S (Hayden et al., 2016).

Salmonella has the ability to resist and survive during commonly used inactivation processes and hostile environments (Li et al., 2013). In addition, *Salmonella* has shown the resistance to many types of stresses including heat, chemical sanitizers, low pH and a cross-protection against multiple stresses in which the exposure to one stress could induce resistance in this organism against other subsequent stresses (Fong and Wang, 2016). Therefore, this ability of *Salmonella* to resist physical and chemical stresses and its widespread incidence in nature present a great food safety challenge for the food industry (Yang et al., 2016). Eggs and poultry are frequently food agents for *Salmonella* infections whereas 13% of *Salmonella* outbreaks are associated with contaminated fresh produce, such as tomatoes, melons, and sprouts (Mba-Jonas et al., 2018)

2.4 Salmonellosis

Salmonellosis, a foodborne illness mainly caused by non-typhoidal *Salmonella enterica* serotypes, is a major public health concern in both developed and developing countries and is commonly identified by a self-limiting gastroenteritis syndrome (revealed as diarrhea, fever, and abdominal pain), with an incubation period between 4 and 72 h and mortality being rare (Antunes et al, 2016). The estimated occurrence of

foodborne illnesses associated with *Salmonella* is the highest among the major bacterial pathogens (Pires et al., 2014). *Salmonella* causes approximately 1.2 million diseases, 23,000 hospitalizations, and 450 deaths in the U. S. every year and food are the source for approximately 1 million of these disease (CDC, 2018c).

In the last decade, fresh fruits and vegetables have been implicated in large outbreaks of human salmonellosis in the U.S. (Table 2.1). Repeated outbreaks of salmonellosis from raw tomatoes, cantaloupes, cucumbers, papayas, and bean, as well as a large-scale outbreak associated with jalapeno and serrano peppers in 2008, emphasized the major challenge to the produce industry and government regulatory agencies in the implementation and consistent application of stringent on farm pathogens control measures (Li et al., 2013). Recently, the U.S. federal government established the Produce Safety Rule (PSR) to regulate fresh produce safety as a part of the Food Safety Modernization Act and the final rule administers staggered sets of compliance dates based on business size (USFDA, 2018b; Bennett et al. 2018). According to the PSR guidance and standards, agricultural water must meet specific standards and corrective actions are required if water quality does not meet these standards (USFDA, 2018b).

There are several factors contributing to the situation above including increasing consumption of fresh produce, changes in production and distribution, and a growing awareness of the problem on the part of public health officials (Lynch et al., 2009). In addition, damaged plant tissues during the precut and prepackage process release nutrients and contribute an appropriate matrix for bacterial growth which increase public health concerns (Li et al., 2013). Moreover, adhesion of the pathogen to surfaces and

Table 2.1. Outbreaks of Salmonellosis associated with fresh produce in the U.S. (2010-2018)

Year	Product	Cases	States	Source
2018	Raw sprouts	10	Multistate	CDC, 2018a
2018	Tomato	14	Kansas	Marler, 2018
2017	Papayas	173	Multistate	CDC, 2018a
2016	Alfalfa sprouts	62	Multistate	CDC, 2018a
2016	Tomato	64	Minnesota	Marler, 2015
2015	Cucumbers	907	Multistate	CDC, 2018a
2015	Tomato	115	Multistate	Murray et al., 2017
2014	Bean sprouts	115	Multistate	CDC, 2018a
2013	Cucumbers	84	Multistate	CDC, 2018a
2012	Cantaloupe	261	Multistate	CDC, 2018a
2012	Mango	127	Multistate	CDC, 2018a
2011	Cantaloupe	20	Multistate	CDC, 2018a
2011	Papayas	106	Multistate	CDC, 2018a
2010	Tomato	51	Florida	Bennett et al., 2015
2010	Alfalfa sprouts	44	Multistate	CDC, 2018a
2010	Tomato	30	Multistate	CDC, 2018a

internalization of the pathogen increases the number of salmonellosis associated with fresh produce due to limitations in the usefulness of conventional processing and chemical sanitizing methods (Lynch et al., 2009; Murray et al., 2017)

Salmonellosis outbreaks due to contaminated fresh produce are commonly large and broadly distributed due to fact that contamination can occur early in the production, such as in the field or in a processing plant (Greene et al., 2008). Bennett et al. (2015) reported that salmonellosis outbreaks were associated with tomatoes probably contaminated on the farm including irrigation water or in the washing water steps in the packinghouse. Greene et al. (2008) indicated that *S.* Newport strain caused disease in at least 72 patients in 16 states due to consumption of contaminated tomatoes during 2005 and the outbreak strain was isolated from pond water used to irrigate tomato fields. Recently, tomatoes contaminated with *S.* Typhimurium included 190 cases of illness and 24 hospitalizations in 2006, where the outbreak strain was traced back to a packinghouse in Ohio (Bennett et al., 2015). More recently, tomatoes contaminated with *S.* Newport grown in Florida were involved in a multistate salmonellosis outbreak that sickened 65 people and hospitalized 11 people in 2007. Some other salmonellosis outbreaks associated with raw tomatoes in the U.S. are presented in Table 2.1.

Zhuang et al. (1995) described the mechanisms of contamination of tomatoes in the field and showed that if dilated plant cells on the surface of warm tomato were exposed to cold water contaminated with *Salmonella*, the cells of tomato would rapidly contract and take in the *Salmonella* through openings such as steam scar. In addition, many studies have found that *Salmonella* may enter tomato plants through roots,

flowers, leaves, stem, scars, small cracks, in the fruit`s skin, or wounds on the plant to contaminate the internal flesh of tomato fruits (Barak and Liang, 2008; Hanning et al., 2009; Gu et al., 2011; Gurtler et al., 2018). Bennett et al (2015) analyzed the information available from environmental investigations (n = 12) regarding salmonellosis outbreaks linked to tomatoes at farms and reported that there were several potential points of contamination, proliferation, and amplification for *Salmonella* on tomatoes due to the use of surface water for irrigation and application of chemicals to tomato plants (n = 7), presence of wild, such as reptiles, birds, and rodents, or domesticated, such as cattle, animals or their feces in tomato fields or in adjacent wild animal habitats or pastures (n = 4), and location of tomato fields in low-lying, flood-prone areas (n = 2). For instance, Iturriaga et al. (2007) determined that based on the environmental conditions, *S.* Montevideo inoculated on the surface of tomato could attach to the exterior of the tomato and populations increased by 0.7, 1.0, 1.2, and 2.2 log CFU per tomato at 60, 75, 85, and 97% relative humidity (RH), respectively, after 10 days of storage at 30°C. Once contamination occurs, it can be difficult eliminate/remove *Salmonella* on tomatoes because of the inadequate efficacy of chlorine treatments.

2.5 Factors affecting fresh produce contamination

Because of the open nature of the production chain, fresh produce can become contaminated with pathogens including *Salmonella* spp. from multiple sources. Several studies have identified the source of contamination occurring through the water, soil, air, biological amendments, wind, and activity of wild animals (Lynch et al., 2009; Berger et al., 2010; Olaimat and Holley, 2012; Goodburn and Wallace, 2013, Meireles et al.,

2016). Preventing contamination during growing and harvesting is the best approach, but this is not always possible (Ramos et al. 2013). For instance, the application of Good Agricultural Practices (GAPs) can be implemented to decrease the risk of preharvest produce contamination (Van Boxstael et al., 2013). Nevertheless, pathogenic microorganisms can still survive for extended durations and become broadly distributed (Murray et al., 2017).

2.5.1 Irrigation Water

Irrigation water can be directly drawn from natural sources such as rivers, lakes, rainwater, groundwater captured in wells, reclaimed wastewater, or potable water sources (FAO/WHO, 2008). Irrigation water can be a vector for pathogenic microorganisms including *Salmonella, E. coli*, and *L. monocytogenes* and is cited as a major potential risk factor for contamination of fresh produce (Doyle and Erickson, 2008; Hanning et al., 2009; Pachepsky et al., 2011; Gil et al., 2015). Duffy et al. (2005) found that 16 of the total 170 (9.4%) *Salmonella* isolates were obtained from irrigation water samples in Texas. Accordingly, a study pointed out that *Salmonella* spp. *Campylobacter* spp. and Enterohemorragic *E. coli* (EHEC) isolates were more often obtained from irrigation water sampled from open field farms (21/45, 46.7 %) versus from greenhouse production (9/75, 12.0 %) (Holvoet et al., 2014). These pathogens in water can then be transmitted to fresh produce during irrigation.

More than 50% of farms in the U.S. apply irrigation using groundwater from wells (Pradhan et al., 2018). The microbiological quality of groundwater may be influenced by depth to the groundwater and it usually improves with distance below

surface because when the distance from surface to groundwater table increases the travel time for pathogens to die off and/or be filtered before reaching the groundwater system (Gerba, 2009; Pachepsky et al., 2011). Nevertheless, pathogens can be found in shallow aquifers and wells (Borchardt et al., 2003; Duffy et al., 2005; Uyttendaele et al., 2015; De Giglio et al., 2016).

Irrigation water from surface water such as streams, rivers, lakes, and ponds may be a source of microbial contamination in fresh produce due to contamination from livestock, wildlife habitat, and humans and their wastes (Leifert et al., 2008; Pachepsky et al., 2011). Animal feces are the primary origin of pathogens in the irrigation water drawn from surface water (Pradhan et al., 2018). Many studies show that grazing cattle and livestock production influenced the quality of surface water and were implicated in contamination of a variety of fresh produce with pathogen (Johnson et al., 2003; FAO/WHO, 2008; Rzezutka et al., 2010). In addition, there are many indirect routes, such as runoff from fields or farms, runoff from manure and pasture lands, overflow from manure lagoons, discharge of raw sewage or wastewater from sewer lines, and subsurface flow or leakage from defective septic systems, can cause the contamination of surface water (FAO/WHO, 2008; Uyttendaele et al., 2015).

The type of irrigation water including overhead sprays, drip irrigation systems, or flooding of fields through furrows has an impact on the transmission of pathogens from irrigation water to fresh produce. Spray and flood irrigation may directly deliver contaminated water onto the edible leaves of fresh produce, thus increasing the risk of contamination. Solomon et al. (2002) reported that 90 % of lettuce plants spray-irrigated

with water containing 7 log CFU/ml of *E. coli* O157:H7 were contaminated, whereas only 19% of plants were contaminated when surface irrigation was used. The authors also found that after treatment of harvested lettuce plants with 200 ppm chlorine, 73 % and 6 % of plants were still positive when spray and surface irrigation were applied, respectively. Stine et al. (2005) found that the level of *Salmonella* contamination in cantaloupe and iceberg lettuce was higher for furrow irrigation compared with subsurface drip irrigation methods.

2.5.2 Manure and soil amendments

Organic fertilizers such as animal manure can be contaminated with pathogen microorganisms and promote the survival or proliferation of these pathogens in the environment and on crops (FAO/WHO, 2008). These fertilizers play an important role in the production of fresh produce because they provide organic matters and nutrients and help improve overall soil quality. Park et al. (2014) reported that 795 out of 955 farmers used manure and 60% of them used cattle manure and may studies determined that *Salmonella* and *E. coli* were isolated from manure and their prevalence varied with the animal of origin (Lejeune et al., 2001; Kunze et al., 2008; Gould et al., 2017). Prevalence of *Salmonella* in healthy cattle feces in different seasons on the same farm in the southwest region of the U.S range from 1.7 to 92% (Edrington et al., 2008). Another study collected samples from 19 states (91 dairies and 97 cull cattle between February and July) and reported that *Salmonella* was recovered from 10% of 188 samples in the U.S. (Wasilenko et al., 2014). Therefore, because of the presence of pathogens, manure

can be a potential source of contamination in fresh produce growing in the fields (FAO/WHO, 2008; Pradhan et al., 2018).

The survival capabilities of the pathogens in manure and manure-amended soil can be influenced by temperature, pH, native microflora, fiber content, and aeration (Franz et al., 2008; Shepherd et al., 2007; FAO/WHO, 2008). Human pathogens such as *E. coli* O157:H7 and *Salmonella* may survive for long periods in animal manure at cool temperatures. Semenov et al. (2007) reported that *E. coli* O157:H7 survived in bovine manure for 49 days at 22 or 30 °C and for over 70 days at 5 °C. In addition, a study on the survival of *Salmonella, E. coli* O157:H7, and *Listeria* in 35,000 L tanks of fresh livestock waste (cattle and pig slurries with non-potable water) reported that survival of these pathogens was in the order *Listeria* < *Salmonella* < *E. coli* O157:H7 (Hutchison et al., 2005). Likewise, Nicholson et al. (2005) found that survival of *E. coli* O157:H7, *Listeria*, and *Salmonella* in waste solids ranged from 2 to 32 days and was in the order *Listeria* < *Salmonella* < *E. coli* O157:H7 in solid manure but was in the order *Salmonella* < *Listeria* < *E. coli* O157:H7 in liquid manure and non-potable water. On the other hand, Winfield and Groisman (2003) reported that *Salmonella* spp. are primarily better survivors outside animal hosts and in insect vectors than *E. coli* O157:H7 because they are more resistant to desiccation and exposure to brackish aquatic environments than *E. coli*.

Various studies determined that pathogens can be present and persist on fresh produce grown in manure-amended soil inoculated with high levels of pathogens (Natvig et al., 2002; Islam et al., 2004; FAO/WHO, 2008). In addition, when fresh produce was

grown in inoculated fresh manure-amended soil, enteric pathogens could internalize into the tissue of leaves and increase the risk of contamination of the produce (Solomon et al., 2002; Franz et al., 2008). Furthermore, pathogens present in soil can be transmitted to the fresh produce through harvesting tools during harvest (Yang et al., 2012).

2.5.3 Wildlife and livestock

Contamination of fresh produce with pathogenic microorganisms can occur directly or indirectly via wildlife (e.g., birds, rodents, boars), livestock (e.g., chicken, dogs, cattle), and humans (Hanning et al., 2009; Hilbert et al., 2012; Karp et al., 2015). Many pathogens, such as *Salmonella* and *E. coli* usually associated with fresh produce have been identified from domestic animals and wildlife (Hilbert et al., 2012; Langholz and Jay-Russell, 2013). Foodborne pathogens may be shed into the faeces of infected domestic and wild animals or humans without causing outward signs of illness (FAO/WHO, 2008; Ferens and Hovde, 2011). For example, Gruszynski et al. (2014) tested a total of 262 faecal samples from deer, turtles, and birds collected between November 2010 and July 2011 from seventeen locations on the Eastern Shore of Virginia and found that a total of 23 (8.8%) samples tested positive for *Salmonella* spp. In addition, fruits and vegetables can be contaminated with fecal waste deposited in the field during the growth stage (FAO/WHO, 2008). Most *Salmonella* infections are zoonotic and many animals, such as, poultry, reptiles, cattle, pigs have been shown to harbor this bacterial species (Franz and van Bruggen, 2008; Farias et al., 2015). Forshell and Wierup (2006) estimated that herd prevalence of *Salmonella* in domestic animals ranged between 0% and 90%, based on the animal species and region.

In addition to direct fecal shedding, pathogens such as *Salmonella* and *E. coli* from domestic and wild animals can be delivered indirectly to fresh produce via many routes and vehicles such as rodents, insects, or birds (Skoy et al., 2008; Wales et al., 2010; Hilbert et al., 2012). These carriers can get contaminated from feces of infected hosts and then transfer the pathogens to fruits and vegetables because of their proximity to animal hosts (Liebana et al., 2003; Leffer et al., 2010; Wales et al., 2010).

2.5.4 Climate and weather factor

Climate and whether conditions affect the magnitude and frequency of transfer of pathogenic microorganisms from environmental sources to fresh produce growing in the field (Tirado et al., 2010; Liu et al., 2013). More specifically, the growth and persistence of foodborne pathogens and their transport within the farm environment can be influenced by temperature and precipitation patters (FAO/WHO, 2008).

Temperature is a crucial extrinsic factor for microbial growth and a potential risk factor affecting microbial contamination in produce farms. During warmer months, the prevalence and concentration of pathogens increase in surface water (Haley et al., 2009; Gorski et al., 2011; Gu et al., 2013). The survival and growth of pathogens in manure and manure-amended soil have also increased at high temperature (FAO/WHO, 2008). Natvig et al. (2002) indicated that when manure was applied throughout warm temperature (>20 °C), *Salmonella* and *E. coli* concentrations in soil were higher compared to manure applied in cold months. In addition, *E. coli* and other bacterial indicators were detected frequently in tomatoes and leafy vegetables collected throughout warmer seasons (Ailes et al., 2008; Marine et al., 2015; Pagadala et al.,

2015). Similarly, higher prevalence of *Salmonella* serovars (Agona, Hadar, Heidelberg, Montevideo, Oranienburg, and Typhimurium) was observed from vegetables with manure applied at warmer temperatures (25 °C and 30 °C) while vegetables with manure applied at cold temperatures (-18 °C and 4 °C) were rarely contaminated with *Salmonella* (Holley et al., 2006; Franz and van Bruggen, 2008). Besides, increased temperature can affect the population of insects and pests found in and around produce farms, so the increased activities of these vectors can lead to transfer of foodborne pathogens to produce (Crohn and Bianchi, 2008). Higher temperatures also lead to increased susceptibility of livestock to animal illnesses, which can make them more sensitive to (asymptomatic) colonization by human enteric pathogens (Liu et al., 2013).

Moisture content of soil increases with increased rainfall and increases the survival of pathogens (Beuchat, 2006; Ivanek et al., 2009). Intensive precipitation can raise runoff from surface and subsurface water acting as a transmission agent for pathogens from manure at livestock farms and from grazing pastures (Crohn and Bianchi, 2008; Haley et al., 2009). Heavy rainfall can cause urban wastewater carrying human pathogens to overflow to wells and streams contributing to the dissemination of pathogens in the environment which serves as an agent for transfer of pathogens from soil to fresh produce (Martinez-Urtaza et al., 2004; Gorski et al., 2011; Cevallos-Cevallos et al., 2012).

Wind can also lead to contamination in produce on the field by bringing dust particles onto produce leaves (FAO/WHO, 2008). Studies reported that human pathogens could survive in dust for up to 26 months and 10 months for *Salmonella* and

E. coli, respectively (Davies and Wray, 1996; Varma et al., 2003). Pathogens carried by dust as aerosols can travel long distances with the help of wind (Baertsch et al., 2007). Moreover, alterations in weather can affect the growth and physiological conditions of produce influencing their vulnerability to contamination of pathogens (FAO/WHO, 2008).

2.5.5 Landscape and geographical factor

Landscape and geographical factors such as domestic animal farm, the slope of ground, and forest can pose a risk of contamination for produce and herbs (FAO/WHO, 2008). These factors can favor the presence and the survivability of pathogens and could considerably affect the movement of the pathogens (Pradhan et al., 2018). *Salmonella, L. monocytogenes*, and *E. coli* survived in the clay loam grassland soil in the presence of manure up to 100 days at Nottinghamshire, UK (Nicholson et al., 2005).

Location of produce-growing farms may impact microbial contamination of produce when adjacent grounds or nearby farms are used for animal husbandry or rendering, such as grazing, housing, feeding, or slaughtering (Crohn and Bianchi, 2008). Strawn et al. (2013) pointed out that *Salmonella* and *L. monocytogenes* were detected in 6.1% and 17.5% of fields in New York State. In addition, the presence of pathogens presence on produce farms can be affected the distance between environmental reservoirs and the farms (Keraita, 2003). Furthermore, the contaminants may be introduced by (i) movement of animals from pasture, feedlot; (ii) movement of animal; (iii) ground sloping toward the crop, contaminated by runoff from rain; and (iv) application of manure on adjacent growing areas (Crohn and Bianchi, 2008). In addition,

Holley et al. (2006) reported that zoonotic pathogens survive longer in moist clay-based soils at lower temperatures in the presence of manure. Farms including animal manure are more probably to be contaminated with enteric pathogens, which can survive in soils for months or years (Olaimat and Holley, 2012).

2.6 Postharvest wash and disinfection methods for fresh produce processing

Figure 2.1 shows a traditional decontamination processing line for fresh produce. Washing is still a crucial step designed to remove dirt, debris, cell exudates after cutting and to decrease field-acquired contamination (Barrera et al., 2012; Li et al., 2017). Proper washing may decrease microbial and chemical contaminants to protect produce wholesomeness, industrial profit, and public health whereas improper washing may accelerate spoilage, product recall, and/or foodborne disease (Gil et al., 2009; Manzocco et al., 2015).

The washing step is a potential pathway for cross-contamination between contaminated and uncontaminated produce in the washing tank because of dispersion of pathogenic microorganisms (Holvoet et al., 2012; Lopez-Galvez et al., 2018). Washing with potable water has limited efficacy (0.5-2.0-log reduction) to remove naturally present microorganisms on fresh produce due to microbial attachment to surfaces, biofilm formation, or microbial internalization into plant tissues (Warriner et al., 2009; Gombas et al., 2017; Murray et al., 2017). In addition, water can play a role for internalization of foodborne pathogens into fresh produce (Zhuang et al., 1995; Buchanan et al., 1999; Gomez-Lopez et al., 2013). Xia et al. (2012) found that *S. enterica* bacteria internalized into the core tissue segments immediately

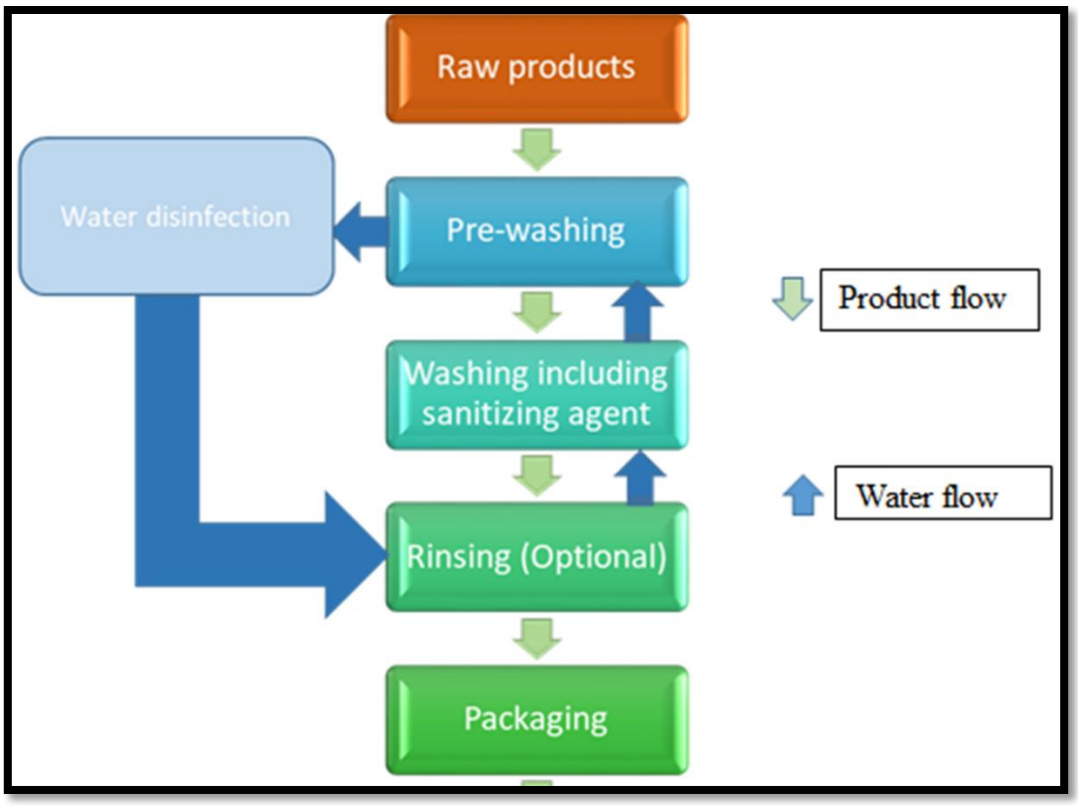

Figure 2.1. Traditional decontamination processing line for fresh produce

underneath the steam scars when mature green tomatoes at 32.2 °C were immersed in water containing about 10^6 CFU/ml *S. enterica* serovar Thompson. Therefore, effective sanitizing strategies focusing on preventing cross-contamination in the washing tank must be developed as opposed to decontaminating produce directly (Gil et al., 2009; Van Haute et al., 2015; Banach et al., 2017).

Water disinfection is applied to maintain the water quality during produce processing and minimize cross-contamination (Meireles et al., 2016; Banach et al., 2015; Millan-Sango et al., 2017). Water reuse or recirculation is a common procedure in the fresh produce industry for water conservation and reduction of operational cost (Gil et al., 2009). During the washing process, the increasing presence of organic and inorganic matters in the wash water is what typically causes water quality degradation, characterized by a measured increase in turbidity (NTU), chemical oxygen demand (COD), and decrease in effective sanitizer concentration (Luo et al., 2012; Weng et al., 2016; Lopez-Galvez et al., 2018). The initial was water turbidity and COD in a commercial washing system were measured as 0.5-0.6 NTU and 301-366 mg/l, respectively, before the introduction of 1620 kg shredded iceberg lettuce and spinach into the system. After washing the produce, measured values were 24 NTU and 1374 mg/l, respectively, (Luo et al., 2012).

The commercial washing process for tomatoes is similar to that used for leafy greens (Zhou et al., 2014a). The tomatoes are discharged from bins into dump tanks where sanitized water is used as a cushion to avoid mechanical damage (Bartz et al., 2015). Zhou et al. (2014) showed that most packinghouse reuse and recirculate the water

in dump tanks. A number of studies reported that the water quality in these dump tanks quickly declined with accumulation of soil, leaf debris, dust, waxes, pesticide residues, and fruit exudates of damaged fruits (Bartz, 2001; Tomas-Callejas, 2012; Huang et al., 2018a). In addition, Zhou et al. (2014) determined that water quality continuously deteriorated during packinghouse operations and the fruit-loading-rates correlated with a significant rise in total dissolved solids (TDS), turbidity, and COD over time.

Water reuse and recirculation can result in the build-up of microbial loads, including pathogens from the crops (Lopez-Galvez et al., 2018). Thus, disinfectant agents should be used to maintain the water quality and prevent cross-contamination of the product despite their limited direct antimicrobial benefit on the produce (Murray et al., 2017; Gil and Allende, 2018). In contrast, wash water quality parameters, such as hardness, pH, COD, and dissolved organic carbon (DOC) correlates with sanitizing agents, such as chlorine, demand and hence with sanitizing agent replenishment (Driss and Bouhelassa, 2014; Gomez-Lopez et al., 2017; Huang et al., 2018b; Van Haute et al., 2018). Banach et al (2017) showed that pathogen inactivation in lettuce wash water was dependent on the organic load of water, temperature, and pathogen attachment and release from the produce.

In conclusion, the water disinfection step in fresh produce processing is critical for removal, inactivation or control of pathogens and other microorganisms because the produce is probably consumed raw and without heat treatment (Meireles et al., 2016). Several chemical (e.g., chlorine and H_2O_2) and physical (e.g., irradiation and plasma) methods are used to reduce the population of these microorganisms in fresh produce.

2.6.1 Chemical methods

2.6.1.1 Chlorine

Chlorine is broadly used to sanitize produce, contact surfaces, and facilities as well as to diminish microbial loads in water used during cleaning and packaging because of its comparably cheap, facility to apply and wide spectrum of antimicrobial effectiveness (Meireles et al. 2016). It is used as hypochlorous acid and hypochlorite as a sanitizing agent at concentrations between 50 and 200 ppm of free chlorine. Typical contact times are less than 5 min (Goodburn and Wallace 2013; Ramos et al., 2013; Chen and Hung, 2016). Generally, pH values between 6.0 and 7.5 are used to maintain high antimicrobial efficacy and minimize corrosion of equipment (Van Haute et al., 2013).

The efficacy of chlorine to reduce microbial loads is limited and ranges from <1 log CFU/g to 3.15 log CFU/g, depending on inoculation method, chlorine concentration, contact time, pH, temperature, and the target bacteria (Goodburn and Wallace, 2013; Ramos et al. 2013; Murray et al., 2017). Beuchat et al. (1998) pointed out that an approximate 1.4-log reduction in the number of *S.* Montevideo on tomato surface occurred when tomatoes were dipped in 320 ppm of active chlorine solution for 2 min. Recently, it was reported that chlorine treatment (200 ppm at 35 °C) for 60 and 120 s reduced *Salmonella* (Agona, Gaminara, Michigan, Montevideo, and Poona) cell numbers inoculated at stem scar on tomatoes by 1.65- and 2.53-log reduction, respectively, and while unsanitized controls only had 1.18- and 1.27-log reduction, respectively.

The efficacy of chlorine to inactivate *E. coli* O157:H7 under conditions simulated commercial operations for the production of fresh-cut leafy greens decreased with changes in total solids, COD, turbidity, and maximum filterable volume (Davidson et al., 2013). Maintaining a constant chlorine concentration to ensure water quality and avoid cross-contamination throughout commercial fresh produce processing is a difficult technical challenge due to lack of monitoring and detecting of dose and other critical quality parameters (Gombas et al., 2017; Gil and Allende, 2018). Furthermore, chlorine reacts with organic matter accumulating in wash water during industrial washing processes resulting in the formation of chlorinated by-products (DBPs), such as trihalomethanes (THMs) and haloacetic acids (HAAs), with potential adverse health effects (Olmez and Kretzschmar, 2009; Ramos et al., 2013; Van Haute et al., 2013). In addition, the use of chlorine in ready to use (RTU) products is prohibited in some European countries including Germany, Holland, Switzerland, and Belgium and future regulatory restrictions are likely due to its environmental and public health impacts. Hence, there is a need to delevop other functional alternatives (Rico et al., 2007; Meireles et al. 2015; Meireles et al., 2016).

2.6.1.2 Chlorine dioxide

Chlorine dioxide (ClO_2) was approved by the FDA in 2001 for use in fresh produce to reduce or eliminate pathogens such as *Salmonella* and *E. coli* (Kaye et al., 2005; Keskinen and Annous, 2011). This compound has effective biocidal activity over a wide range pH (3-8) and can be produced *in situ* by the reaction of an acid with sodium chlorite, or the reaction of sodium chlorite with chlorine gas (Gomez-Lopez et al., 2009;

Saade et al., 2017). Compared to chlorine, ClO_2 is less corrosive (Olmez and Kretzschmar, 2009), inhibits enzymatic browning (Chen et al., 2010), has a higher oxidation capacity and lower reactivity with organic matter (Tomas-Callejas et al., 2012), and it does not react with nitrogen or ammonia to form harmful by-products (Rico et al., 2007). Nevertheless, its use has many disadvantages including being readily degraded when exposed to sunlight, it is explosive, and not very effective a maximum allowed concentration (up to 3 ppm) (Olmez and Kretzschmar, 2009; Ramos et al., 2013; Meireles et al., 2016).

The pathogen inactivation efficacy of ClO_2 on fresh produce has been evaluated (Mahmoud and Linton, 2008; Sun et al., 2017; Bridges et al., 2018). Mahmoud and Linton (2008) demonstrated that approximately 0.5, 0.9, 1.2, 1.6, and 1.6 log CFU/5 cm^2 and 0.7, 0.7, 1.0, 1.2, and 1.5 log CFU/5 cm^2 reductions of *E. coli* O157:H7 and *Salmonella* serovars (Enteritidis, Javiana, and Montevideo), respectively, were achieved by treatment of inoculated on lettuce leaves with 0.5, 1.0, 1.5, 3.0, and 5.0 ppm ClO_2 gas at 22 °C and 90-95% relative humidity. Accordingly, the total bacteria count of fresh-cut cucumber, lettuce, carrots, apples, tomatoes, and guava treated with 100 ppm ClO_2 in aqueous solution decreased by 2.52, 3.5, 3.48, 2.23, 3.93, and 1.52 log CFU/g. More recently, Sun et al. (2017) developed a controlled-release ClO_2 pouch (0.5 g of ClO_2) which exhibited strong antimicrobial activity reducing *E. coli* and *Alternaria alternate* populations on grape tomatoes by 3.08 log CFU/g and 2.85 log CFU/g, respectively, after 14 days of storage at 20 °C. Bridges et al. (2018) found that a 5.0 h ClO_2 exposure resulted in reductions ($\geq$ 7 log CFU/g) below the detection level (< 1 log CFU/g) for *E.*

coli O157:H7, *E. coli* non-O157:H7, and *Salmonella* serovars (Typhimurium, Heidelberg, Enteritidis, Montevideo, and Newport) on tomatoes for both 14 and 30 ppm ClO_2 treatments.

2.6.1.3. Electrolyzed water

Electrolyzed (EO) water is a promising disinfection alternative to chlorine (Huang et al., 2008; Cheng et al., 2012; Meireles et al., 2016). It is classified as acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW) based on the type of electrolyzed water with sanitizing properties (Ramos et al., 2013; Gil et al., 2015). These solutions are formed by electrolysis of diluted sodium chloride (NaCI) solutions (0.5-1.0%) in electrolysis chamber with an anode and a cathode separated by a membrane for AEW and NEW, respectively, (Ramos et al., 2013; Machado et al., 2016; Qi et al., 2018). The AEW and NEW have a strong bactericidal effect with pH values varied from 2.1 to 4.5 and 5.0 to 8.5, respectively, and oxidation-reduction potential values varied from 1000 to 1200 mV and from 500 to 700 mV, respectively, (Graca et al., 2011; Ramos et al., 2013; Gil et al., 2015).

Gil et al. (2015) reported that EO water (i) can be produced on-site; (ii) is environment friendly because of no added chemical, except for NaCI (Bonde et al., 1999); (iii) is quite cost effective because water and NaCI are found virtually everywhere (Venczel et al., 1997); (iv) use reduces the cost of hazards linked to handling, transportation, and storage of concentrated chlorine solutions (Nakagawara et al., 1998; Gomez-Lopez et al., 2017); (v) can be more effective than chlorine to inactivate microorganisms per available chlorine concentration (Issa-Zacharia et al.,

2011); (vi) is not only disinfectant but also may prevent enzymatic browning throughout storage of MPV (Koseki and Itoh, 2002); and (vii) can kill microorganisms physically, and that microorganisms do not acquire resistance (Gil et al., 2015). In contrast, organic matter, pH, water hardness, and temperature can greatly affect the efficiency of EO water (Ongeng et al., 2006, Chen and Hung, 2017).

EO water has been used as a decontaminant for fresh produce and disinfectant for the process wash water and food-contact surfaces (Huang et al., 2008; Forghani and Oh, 2013; Afari et al., 2016). Forghani and Oh (2013) showed 1.23 and 1.22 log CFU/g reduction of *E. coli* and *L. monocytogenes* on lettuce, respectively, after treatment with slightly acidic electrolyzed water (SAEW). Accordingly, Ding et al. (2015) indicated that treatment of SAEW for 10 min reduced about 1.45, 0.93, and 1.5 log CFU/g of total aerobic bacteria and 1.10, 0.96, and 1.3 log CFU/g of yeasts and molds on cherry tomatoes, strawberries, and fresh-cut cabbage, respectively. More recently, Afari et al. (2016) found that red round tomatoes treated with deionized (DI) water and near neutral electrolyzed (NEO) water for 5 min reduced the population of *S.* Typhimurium DT 104 by 3.15 and 5.40 log CFU/tomato, respectively.

2.6.1.4 Ozone

Ozone (O_3) is a strong antimicrobial agent generated as gas dissolved in water (Murray et al., 2015). It has high reactivity and penetrability (Ramos et al., 2013; Meireles et al., 2016). The antimicrobial activity of ozone occurs via direct attack by molecular ozone and indirectly by the action of free radicals formed as a result of the decomposition of ozone (Shynkaryk et al., 2015; Tzortzakis, 2016; Pyatkovskyy et al.,

2017). A small concentration (1-5 ppm) of ozone in aqueous solution is adequate for exerting antimicrobial activity, but higher concentration is needed when used as gas because of its poor penetration into cells and the consequent decontamination process is influenced by humidity of the air (Meireles et al., 2016; Pyatkovskyy et al., 2017). Ozone is also environmentally friendly because it quickly decomposes to a non-toxic product, oxygen (O_2), (Meireles et al., 2016).

Ozone is approved by the FDA for the disinfection of fresh produce, process water, and food-contact surfaces (Pyatkovskyy et al., 2017; Bridges et al., 2018). Hirneisen et al. (2011) stated that after 5 min of ozone (6.25 ppm) treatment, human norovirus surrogates, feline calicivirus (FCV) and murine norovirus (MNV), were inactivated as 6.79 and 4.69 log $TCID_{50}$ (50% tissue culture infectious dose)/ml, respectively, in water and 2.09 and 2.91 log TCID50/ml, respectively, on lettuce. Bermudez-Aguirre and Barbosa-Canovas (2013) also demonstrated that a 15 min of ozone (5ppm) treatment reduced the concentration of *E. coli* on grape tomatoes by 2.2 log CFU/g.

The use of ozone presents some disadvantages such as the compound is unstable and corrosive to equipment, it is sensitive to the presence of organic matter, and it may have negative impact on the sensory characteristics of the product (Chawla et al., 2012; Pyatkovskyy et al., 2017). The efficacy of ozone treatment against pathogenic microorganisms on fresh produce can be affected by type of fresh produce, microorganism, initial inoculum level, incubation conditions, ozone delivery method, concentration and time of exposure, and other environmental factors including

temperature, pH, and organic load of treatment medium (Glowacz et al., 2015; Yesil et al., 2017)

2.6.1.5 Organic acids

Organic acids are natural or chemically synthetized sanitizing agents commonly used in the food industry. USFDA and the European Commission (EC) approved the use of lactic, citric, acetic, ascorbic, and tartaric acid, as antioxidants, flavoring agents, and preservatives, among other applications (Meireles et al., 2016). These compounds are used in fresh produce industry due to their strong antimicrobial activities and their presence may disturb membrane transport and/ or permeability, anion accumulation, and a reduction in internal cellular pH (Ramos et al., 2013; Linares-Morales et al., 2018). Park et al. (2011) reported that after 10 min of treatment with 1 % and 2 % organic acids against *E. coli O157:H7, S.* Typhimurium, and *L. monocytogenes* on lettuce, propionic (0.93 to 1.52 log reduction), acetic (1.13 to 1.74 log reduction), lactic (1.87 to 2.54 log reduction), malic (2.32 to 2.98 log reduction), and citric acid (1.85 to 2.86 log reduction) showed significant ($P < 0.05$) effects compared to the control treatment (distilled water). Nevertheless, the use of high concentration of organic acids may affect the organoleptic qualities of fresh produce, corrosive the processing equipment, and increase the cost of the process due to their initial high cost (Sagong et al., 2011; Meireles et al., 2016).

2.6.1.6 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) consists of two atoms of hydrogen (H) combined with oxygen (O_2). H_2O_2 has been studied as an alternative to conventional chlorine sanitizers for decontaminating fresh produce because it is colorless, non-corrosive,

environmentally friendly, and it quickly decomposes into water and oxygen in the presence of catalase as well as it does not react with organic compounds present in perishables which may produce carcinogenic compounds (Meireles et al., 2016). H_2O_2 is classified as generally regarded as safe (GRAS) for use in food products as a bleaching, oxidizing and reducing, and antimicrobial agents (Raffellini et al., 2008).

Hydrogen peroxide has both bacteriostatic and bactericidal activity because of its strong oxidizing power and the generation of cytotoxic agents, such as hydroxyl radical (Olmez and Kretzschmar, 2009; Ramos et al., 2013). Imlay et al. (1988) reported that exposure of *E. coli* to low concentrations of H_2O_2 resulted in DNA damage that causes mutagenesis and kills the bacteria, while higher concentrations of H_2O_2 decrease the amount of such damage due to its suppression of DNA damage. The authors indicated that the major portion of the toxicity of hydrogen peroxide in *E. coli* was attributed to DNA damage mediated by a Fenton reaction that produces active forms of hydroxyl radicals from hydrogen peroxide, DNA-bound iron, and a constant source of reducing equivalents.

Ukuku and Fett (2002) found that the population of *L. monocytogenes* on the surface of melons, rotating by hand to complete coverage and contact of surfaces with a 5% H_2O_2 wash solution for 2 min, decreased by 2.0 – 3.5 log CFU/cm^2. Accordingly, Lin et al. (2002) reported that the populations of *L. monocytogenes, Salmonella* Enteritidis, and *E. coli* O157:H7 on lettuce treated with 2% H_2O_2 at 50°C for 90 s decreased by 2.7, 4.5, and 4.7 log CFU per leaf, respectively. Recently, Huang and Chen (2011) achieved 2.2 log CFU/g reduction of *E. coli* O157:H7 on spinach leaves treated

with 2% H_2O_2 in deionized water at 50 °C. Similarly, Huang et al. (2012) used 3% H_2O_2 in deionized water at 22 °C and achieved 1.6 log CFU/g reduction of *E. coli* O157:H7 on spinach leaves for 5 min. More recently, Guo et al. (2017) found that the concentration of *Salmonella* cocktail inoculated on grape tomatoes washed with 1% H_2O_2 for 2 min at 30 °C decreased by 2.41 log CFU/g while they reported *Salmonella* survivors in wash water.

Several studies have evaluated the effect of hydrogen peroxide on the quality of fresh produce (Ukuku, 2004; Jiang et al., 2017; Islam et al., 2018). For instance, appearance and overall acceptability rating for fresh-cut cantaloupe pieces obtained from whole cantaloupes treated with 1% H_2O_2 were higher ($P < 0.05$) than that for untreated samples after 15 days of storage at 5 °C (Ukuku, 2004). Similarly, no significant differences ($P > 0.05$) were detected in firmness to touch, color, and aroma/smell intensities of strawberries rinsed in hydrogen peroxide at 1% (Alexandre et al., 2012). Recently, Jiang et al. (2017) reported that color and texture of grape tomatoes, baby spinach leaves, and cantaloupes were not influenced ($P > 0.05$) by the aerosolized H_2O_2 (7.8%). More recently, Islam et al. (2018) found that a 5 mg/L of hydrogen peroxide sprayed to tomato plants did not affect ($P > 0.05$) the texture, color, lycopene, titratable acidity, vitamin C, soluble solids, fructose, and glucose contents of cherry tomatoes after 20 days of storage at 5 °C.

2.6.1.7 Miscellaneous chemical methods

The number of chemical sanitizers with the potential application to decontaminate fresh produce has increased in the last decade after concerns regarding

the safety of chlorine. Yuk et al. (2005) demonstrated that all aqueous sanitizing treatments, 200 ppm chlorine, 1200 ppm acidified sodium chlorite (ASC), and 87 ppm peroxyacetic acid (PAA) at 35 °C for 60 and 120 s achieved over 4.0, 1.0, and 2.0-log reductions of *Salmonella enterica* serovars (Agona, Gaminara, Michigan, Montevideo, and Poona) on smooth surface, stem scar, and wounds of green tomatoes ('Florida 47' cultivar), respectively. Accordingly, Neal et al. (2012) tested the effectiveness of multiple chemical sanitizers including 2 % lactic acid at 55 °C, peroxyacetic acid (80 ppm), calcium hypochlorite (200 ppm), ozonated water (1.2 ppm), and chlorine dioxide gas (ClO_2, 2.1 ppm) on reduction of *Salmonella* spp. and *E. coli* O157:H7 on spinach and found that lactic acid produced a 2.3 log CFU/g reduction for *Salmonella* and a 2.7 log CFU/g reduction for *E. coli* O157:H7, higher ($P < 0.05$) than other treatments. More recently, Petri et al. (2015) found that population of *E. coli* O157:H7 on fresh-cut lettuce were decreased ($P < 0.05$) by 1.28, 1.41, 2.21, and 2.49 log CFU/g after washing with tap water, ClO_2, PAA, and chlorine. In conclusion, all these chemical sanitizers described above display limited efficiency in reducing microbial loads (< 5.0 log-reduction) on fresh produce (Meireles et al. 2016).

Peroxyacetic acid (PAA) and acidified sodium chlorite (ASC) have been tested in their effectiveness to reduce microbial contaminations associated with fresh produce (Ramos et al., 2013; Meireles et al., 2016; Murray et al., 2017). PAA is the peroxide of acetic acid (AA) and a strong oxidant and disinfectant (Olmez and Kretzschmar, 2009; Olaimat and Holey, 2012). The oxidation potential of PAA is larger than that of chlorine or ClO_2 and is commercially available in the form of a quaternary equilibrium mixture

including AA and H_2O_2, and water (Abadias et al., 2011). PAA has been approved by the FDA for sanitizing certain food products, including fruit and vegetables, at concentrations that do not exceed 80 ppm in wash water (Vandekinderen et al., 2009). The disinfection efficiency of PAA toward microorganisms may be classified in a system as follows: bacteria > viruses >bacterial spores > protozoan cysts (Vandekinderen et al., 2009). Park and Beuchat (1999) showed that PAA at concentration of 40-80 ppm reduced the population of *Salmonella* and *E. coli* O157:H7 on the surface of cantaloupe and honeydew melon in the range of 2.6-3.8 log CFU/g. A study carried out by Fan et al. (2009) found that treatments with 180 ppm of chlorine, 1200 ppm acidified calcium sulphate (ACS), 1000 ppm of acidified sodium chlorite (ASC), 80 ppm of PAA, and a combination of ACS (1200 ppm) and PAA (80 ppm) for 10 min had limited effect on *S.* Poona population no more than a 1.5 log reduction on the surface of whole cantaloupes. Zudaire et al. (2018) pointed out that peracetic acid (80 ppm) for 5 min achieved a 0.79 log CFU/g reduction for total mesophilic aerobic count and a 0.78 log CFU/g for yeast and molds on fresh-cut calcots (*Allium cepa* L.,).

Acidified sodium chlorite (ACS) was recently approved by the FDA for spraying or dipping treatments in the range of 500 to 1200 ppm for fresh produce decontamination (Ramos et al., 2013). ACS is a substantial antimicrobial produced by lowering the pH (2.5-3.2) of solution of sodium chlorite with any GRAS acid producing chlorous acid (Artes et al., 2009). ASC at 1200 ppm reduced *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* inoculated on leafy greens by 3.6, 3.8, and 3.0 log CFU/g, respectively, without adversely affecting the physical appearance of the leaves

(Stopforth et al., 2008). Allende et al. (2009) observed that a maximum reduction of *E. coli* O157:H7 population on fresh-cut cilantro (>3 log CFU/g) was achieved after washing the produce with 1000 ppm of ASC.

2.6.2 Physical non-thermal decontamination methods

2.6.2.1 Ultraviolet light

Ultraviolet (UV) light is classified as UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm), and vacuum UV (100-200 nm) based on the wavelength of electromagnetic radiation (Ramos et al., 2013; Meireles et al., 2016). The UV-C light is useful for surface treatment and has a wide spectrum of microbicidal action (Yaun et al., 2003; Guo et al., 2017). Microbial inactivation effectiveness is highly correlated with the UV energy dose received (Selma et al., 2008; Adhikari et al., 2015). At least 400 J/m^2 doses of UV is needed to achieve microbial inactivation including pathogens (Sastry et al., 2000). The microbial inactivation mechanism of UV light is the damage to DNA which lead to cell death (Ge et al., 2013). UV treatment is easy to use and is cheap, lacks residues, and is environmentally friendly (Meireles et al., 2016; Murray et al., 2017). However, the efficacy of UV treatment is limited to surfaces and transparent liquids since UV light is absorbed by food surfaces and also increases temperature which may damage the produce (Li and Farid, 2016).

The advantage of using UV for decontamination of fresh produce and water disinfection has been widely studied (Yaun et al., 2004; Mukhopadhyay et al., 2014 and 2015; Guo et al., 2017). Yaun et al. (2004) investigated that the bactericidal effect of UV-C (253.7 nm) on the surface of leaf lettuce and tomatoes inoculated with cultures of

Salmonella enterica serovars (Montevideo, Agona, Baildon, Michigan, Gaminara) or *E. coli* O157:H7. The authors found that maximum log reductions on green leaf lettuce for *Salmonella* and *E. coli* O157:H7 seen at dose of 24 mW/cm^2 which were 2.65 and 2.79 log CFU/lettuce, respectively. They also determined that UV-C was less effective at reducing populations of *Salmonella* on the surface of tomatoes when compared to green leaf lettuce and it achieved a maximum log reduction of 2.19 log CFU/tomato at doses of 24 mW/cm^2. In addition, Mukhopadhyay et al. (2014) reported that a dose of 6.0 kJ/m^2 of UV-C at room temperature and relative humidity of about 60% resulted in 3.49 log CFU/tomato reduction of *E. coli* O157:H7 compared to 3.06 log CFU/tomato reduction of *Salmonella* spp. on the surface of grape tomatoes. Mukhopadhyay et al. (2015) showed that a low dose of 0.6 kJ/m^2 of UV-C at room temperature and relative humidity of about 60% provided 1.6 log CFU/tomato reduction of *Salmonella* spp. inoculated on the surface of plum tomatoes. Guo et al. (2017) investigated the effect of three forms of UV treatment (~29 mW/cm^2), dry UV (samples were treated by UV directly), wet UV (samples were dipped in water briefly and then exposed to UV), and water-assisted UV (samples were treated by UV while being immersed in agitated water) on inactivation of *Salmonella enterica* serotypes (Montevideo, Newport, Saintpaul, and Stanley) inoculated on grape tomatoes for 2 min. The authors found that the water-assisted UV treatment was the most effective for decontamination of tomatoes since it reduced the population of *Salmonella* spp. on dip- and spot- inoculated tomatoes by 3.84 log CFU/g and 4.97 log CFU/g, respectively. The authors also found that adding chlorine and H_2O_2 into the

water-assisted UV treatment against *Salmonella* spp. on tomatoes did not improve the efficacy of this treatment (Guo et al., 2017).

2.6.2.2 Cold plasma

Plasma is composed of gas molecules dissociated by an energy input (Ramos et al., 2013). The composition of a plasma is broadly connected with gas composition and electric field strength (Ekezie et al., 2017). Cold plasma, the use of non-thermal ionized gases, is an emerging antimicrobial technology. Although cold plasma has been known since the latter part of the 19th century, the need for high voltage generators, excessive heat generation, use of noxious working gases, and requirement for treatment to work under low pressure, has restrained its commercial applications (Murray et al., 2017). Different configurations including partial discharge, dielectric barrier discharge, corona discharge, microwave discharge, and atmospheric plasma jet are used to produce plasma (Pignata et al., 2017).

Cold plasma is comprised by photons, electrons, positive and negative ions, atoms, free radicals, and excited or non-excited molecules having ability to inactivate microorganisms (Ekezie et al., 2017). Its potential as a treatment to inactivate pathogens in fresh produce has been investigated. Treatment of variety fresh produce for 300 s to 20 min can yield a 1-3 log microbial reduction (Pignata et al., 2017). The long treatment times and the fact that the sample must be near the plasma source are major disadvantages of this technology (Murray et al., 2017). In spite of these disadvantages, gas plasma has been produced within modified atmosphere-packed fresh produce where electric discharge is passed through the packaging and by so doing ionizing the gas with

producing antimicrobial radicals *in situ* (Moon et al., 2016). Misra et al. (2014) tested strawberries treated with atmospheric cold plasma (ACP), generated inside a sealed package containing ambient air (42% relative humidity). The authors found that the reduction of total mesophiles and yeast/molds on the surface of strawberries were 2.56 log CFU/g and 1.56 log CFU/g, respectively. Furthermore, after 120 s of atmospheric cold plasma (ACP) treatment, the population of *E. coli*, *Salmonella*, and *L. monocytogenes* inoculated on the surface of strawberries was reduced by 1.6 log CFU/sample, 1.7 log CFU/sample, and 4.2 log CFU/sample, respectively (Ziuzina et al. 2014). An ACP treatment time of 300 s reduced *Salmonella*, *L. monocytogenes*, and *E. coli* counts on lettuce by 2.4, 2.3, and 3.3 log CFU/sample (Ziuzina et al., 2015).

Jiang et al. (2017) inoculated stem scars and the smooth surface of grape tomatoes, spinach leaves, and cantaloupe rinds, with *S.* Typhimurium and treated for 45 s followed by additional 30 min dwell time with cold plasma-activated hydrogen peroxide (H_2O_2; 7.8%) aerosol. The cold plasma-activated H_2O_2 aerosol treatment reduced the population of *S.* Typhimurium on tomato-smooth surface, tomato-stem scar surface, spinach, and cantaloupe by 5.0, 1.3, 4.2, and 1.3 log CFU/piece, respectively, and this treatment did not affect ($P > 0.05$) the color and texture of the produce. More recently, Timmons et al. (2018) observed 1 and 2 log CFU/ml reduction of *S. enterica* serovars (Enteritidis, Typhimurium, Javiana, Seftenburg, and Poona) on the surface of pecans and cherry tomatoes treated with surface dielectric barrier discharge (SDBD) as a cold atmospheric plasma after 4 and 10 min, respectively.

2.6.2.3 Ultrasound

Ultrasound is a form of energy produced by sound waves at high amplitude, and above human-hearing threshold (>20 kHz) (Meireles et al., 2016). Based on the region of the sound spectrum, sound waves are separated into three main parts including infrasound ($v < 16$ Hz), band sound (16 Hz $< v < 16$ kHz), and ultrasound ($v > 16$ kHz) (Arvanitoyannis et al., 2017). The ultrasound band is also separated into low frequency (16 kHz $< v < 1$ MHz) and high frequency ($v > 1$ MHz) bands (Sao Jose et al., 2014). High-frequency ultrasound bands are mainly used in food processing operations to produce emulsions, disrupt cells, and disperse aggregated materials (Demirdoven and Baysal, 2009; Ding et al., 2015; Yu et al., 2016; Huang et al., 2018a).

The efficacy of ultrasound treatment is greatly associated with frequency, the amplitude of ultrasonic waves, exposure time, volume processed, food composition, pH, and treatment temperature (Ananta et al., 2005; Gomez-Lopez et al., 2010; Sao Jose et al., 2014). For instance, the frequency selected for application induces cavitation resulting in bubbles that break down and generate the mechanical energy responsible for the disinfecting action and the chemical energy responsible for the free radical formation, thus increasing the permeability of cell membranes (Bermudez-Aguirre et al., 2011; Sao Jose et al., 2014). The cell shape (coccus bacteria are more resistant), size (smaller cells are more resistant), gram nature (gram positive bacteria are more resistant), and cellular metabolism (aerobic microorganisms are more resistant) also affect the efficacy of ultrasound treatment (Meireles et al., 2016).

High-intensity ultrasound varied from 20 to 100 kHz is effective for produce decontamination and water disinfection (Gomez-Lopez et al., 2015; Arvanitoyannis et

al., 2017; Huang et al., 2018a). Ding et al. (2015) achieved a 0.71 and 0.52 log CFU/g reduction of total aerobic bacteria on cherry tomatoes and strawberries, respectively, treated with ultrasound treatment (40 kHz at 240 W for 10 min). The authors also determined that the total bacteria on the cherry tomatoes was reduced by 1.77 log CFU/g while yeast and molds were reduced by 1.50 log CFU/g after the combined treatment of slightly acidic electrolyzed water (SAEW) and ultrasound. In addition, Afari et al. (2016) found that the inclusion of ultrasound with deionized (DI) water further reduced the *E. coli* O157:H7 population on lettuce leaves by 0.8-1.0 log CFU/g at 130 W and 1.1 to 1.2 log CFU/g at 210 W for 1-15 min. The authors also showed that neutral electrolyzed (NEO) water with ultrasound treatment at 130 W power reduced *S.* Typhimurium DT 104 on round red tomatoes by an additional 1.0 to 1.4 log CFU/tomato after 1- and 5-min treatments, respectively. Furthermore, Millan-Sango et al., (2017) determined that the ultrasound (26 kHz, 90 µm, 41.85 W/L) combined with UV-C light (1.64 kJ/m^2) treatments for disinfection of lettuce wash water after 30 min treatment was the most efficient process tested regarding bacteria inactivation (3.57 log CFU/ml), colour reduction (43.3%), and reduction of suspended particles (30%) when compared to ultrasound and UV-C light applied alone.

2.6.2.4 Advanced oxidation process

Advanced oxidative processes (AOPs) are defined as aqueous phase oxidation methods depending on the intermediacy of highly reactive species such as (primarily but not exclusively) hydroxyl radicals in the mechanisms leading to inactivate bacteria and viruses (Selma et al., 2008; Klavarioti et al., 2009). The hydroxyl radicals have much

more oxidizing power than O_3, H_2O_2, hypochlorous acid, and chlorine (Selma et al., 2008). Production of hydroxyl radicals may be achieved using a variety of methods including heterogeneous and homogeneous photocatalysis based on near ultraviolet (UV) or solar visible irradiation, ionizing radiation, pulsed plasma, electrolysis, ozonation, the Fenton`s reagent, ultrasound, and wet air oxidation (Venkatadri and Peters, 1993; Klavarioti et al., 2009).

The most common AOPs used in fresh produce decontamination to produce hydroxyl radicals is through the use of combined catalytic oxidants such as photocatalysis, O_3-UV, H_2O_2-UV, and H_2O_2-O_3 (Murray et al., 2017). Hadjok et al. (2008) stated that a combination of UV (37.8 mJ/cm^2) and H_2O_2 (1.5%, v/v) at 50 °C for 60 s produced a 4.12, 4.21, and 3.87 log CFU/g reductions of *Salmonella*, *Pseudomonas fluorescens*, and *E. coli* O157:H7 on the surface of iceberg lettuce and a 2.22 and 3.55 log CFU/g reductions of *Salmonella* and *E. coli* O157:H7 on the surface of ripened whole tomatoes. Guan et al. (2013) reported that H_2O_2 (3%)-UV (0.45 kJ/m^2) treatment for 15 s reduced the population of *E. coli* O157:H7 and total aerobic plate counts by 0.87 and 0.24 log CFU/g, respectively, on the surface of mushrooms. Similarly, the log reductions of *L. monocytogenes* on mushrooms exposed to UV (37.8 mJ/cm^2) with misting of 2% (v/v) H_2O_2 at 50 °C for 30 s achieved were less than 0.5 log CFU/g (Murray et al., 2015). More recently, Guo et al. (2017) demonstrated that the combined treatment of water-assisted UV treatment and 1% H_2O_2 reduced the population of *Salmonella* spp. on grape tomatoes by 4.02 log CFU/g. Besides, Selma et al. (2008) found that the 1-min treatment was able to eliminate *E. coli* populations in lettuce and

onion wash waters (4.08 and 3.28-log reductions, respectively) while reductions of only 0.94 and 1.92 log CFU/ml were obtained for chicory and escarole wash waters treated with the heterogeneous photocatalytic system (titanium dioxide (TiO_2) photocatalyst fiber illuminated with a 40-W UV-C lamp). They concluded that *E. coli* inactivation in wash water depended on the quality of the water which is affected by the washed product.

2.6.2.5 Modified atmosphere packaging

Modified atmosphere packaging (MAP) implies changing the gases surrounding a commodity to produce a composition different from that of air created either passively, by product respiration and film permeability to attain the desired gas a composition over time (passive MAP), or with intent, by substituting air with a proper gas mixture in the package (active MAP) (Rico et al., 2007; Caleb et al., 2013; Wilson et al., 2017).

The MAP system can protect fresh produce against deteriorative effects, such as discoloration, off-flavor and off-odor development, nutrient loss, and texture, and inhibit growth of pathogens (Zhang et al., 2015). Thus, the proper MAP system can retain the overall quality of fresh produce if the optimum gas composition and permeability of the film are designed correctly (Belay et al., 2016). Sant`Ana et al., (2012) studied the efficacy of MAP systems (5% O_2 and 15% CO_2 and 80% N_2) on Salmonella spp. inoculated in green salad (crisp, romaine, butter lettuce, cabbage, escarole, collard green, spinach, watercress, arugula, grated carrot, mix for yakisoba, (broccoli, cabbage, cauliflower, leek, carrots, chard) for 6 days at 7 °C. The authors found that the pathogen was only able to grow in escarole (approximately 1.0 log CFU/g) and arugula

(approximately 2 log CFU/g) while its population decreased in cabbage and carrots. Oliveira et al. (2015) reported that an atmosphere of 3-6% O_2 and 2-10% CO_2 achieved microbial control and extended the shelf-life of a wide variety of fresh-cut products. Dominguez et al. (2016) demonstrated that the MAP system had a positive effect on the content of lycopene, ascorbic acid, and total phenols of tomato fruits

Current MAP systems alone cannot be enough to effectively prevent deterioration and decrease microbial growth in fresh produce (Wilson et al., 2017). Hence, many researchers have focused on examining potential synergistic effects of MAP with other post-harvest sanitation technologies including UV-C and edible films. The combination of MAP (2-10 kPa O_2 + 5-12 kPa CO_2 steady state) and UV-C radiation reduced the growth of psychotropic, coliform bacteria, and yeast on fresh processed lettuce (Allende and Artes, 2003). Similarly, UV-C combined with active MAP (10 kPa O_2 + 10 kPa CO_2) controlled the growth of natural microbiota on strawberries after 12 days of storage (Allende et al., 2007).

Das et al. (2006) demonstrated that during MAP (4% CO_2 + 6% O_2), the population of *S.* Enteritidis on the surface of cherry tomatoes decreased by 4.0-5.0 log CFU/tomato but the growth (approximately 1.0 log CFU/tomato) was observed in stem scars of cherry tomatoes after 20 days of storage at 7 °C. In addition, the treatment with MAP (100% CO_2) following treatment with ClO_2 during 7 days of storage at 7.0 °C reduced the population of *E. coli* O157:H7 on spinach by 3.9 log CFU/g (Lee and Baek, 2008). Furthermore, the combination of MAP (5.3% CO_2 + 5.5% O_2) and UV-C irradiation achieved a 2.17 log CFU/g reduction of *S.* Typhimurium inoculated to the

cherry tomatoes after 9 days of storage at 4 °C whereas the combination of these treatment did not affect ($P > 0.05$) the population of *S*. Typhimurium on the cherry tomatoes after 9 days of storage at 20 °C (Choi et al., 2015).

The effect of a sodium alginate edible coating enriched with active compounds (hydro-alcoholic-solution and grape seed extract) increased the shelf-life of the minimally processed kiwifruits up to 14 and 12 days when packed in two MAP conditions (passive and low oxygen atmosphere with 10 kPa O_2 + 10 kPa CO_2, respectively) compared to 8 days for the control samples (Mastromatteo et al., 2011). Accordingly, Guimaraes et al. (2016) determined that a starch coating reinforced with natural smectite montmorillonite nanoparticles and passive MAP (15 kPa O_2 + 20 kPa CO_2 steady state) led to the preservation of the total antioxidant activity the volatile and organic acids of minimally processed carrots.

2.6.2.6 High-pressure processing

High pressure processing (HPP), described as the utilization of elevated pressures, mainly in the range of 100-700 MPa, is regarded as a promising alternative to thermal treatment, in terms of safety assurance and minimal effects on produce quality (Murray et al., 2017; Huang et al., 2017). HPP ensures a unique advantage since pressure acts uniformly during the whole of a food regardless shape, size, and geometry (Lou et al., 2012), and has minimal effects on the texture, taste, flavor, and appearance (Oey et al., 2008; Tewari et al., 2017). Although HPP has been studied very well in the non-thermal pasteurization of juices, purees, deli meats, and seafood (Possas et al., 2017; O`Neill et al., 2018; Ribeiro et al., 2018), the application to fresh produce is rare because

the high pressure short time treatment needed for inactivation of foodborne bacteria and viruses negatively affects the produce quality (Torres and Velazquez, 2005; Murray et al., 2017). Woolf et al. (2013) pointed out that HPP treatment of 600 MPa for 10 min extended the shelf-life of avocado slices up to 60 days but affected ultrastructure and induced undesirable color changes.

Maitland et al. (2011) showed that significant reductions of *S.* Braenderup concentrations in diced tomatoes ($P < 0.05$) were achieved after processing with HPP at 350 MPa (0.46 CFU/g), 450 MPa (1.44 log CFU/g), and 550 MPa (3.67 MPa) at 20 °C for 120 s. The authors also reported that significant reductions ($P < 0.05$) of this pathogen were achieved on whole tomatoes packaged in $CaCl_2$ treated at 350 MPa (1.41 log CFU/g) 450 MPa (2.25 log CFU/g), and 550 MPa (3.35 log CFU/g) at 9.49 °C to achieve an end-processing temperature of approximately 20 °C for 120 s without changes in the tomatoes visual appearance.

HPP has been widely evaluated to inactivate pathogens in puree-type foods and fruits and vegetables juice (Huang et al., 2013; Mukhopadhyay et al., 2016; Shahbaz et al., 2016). The population of *E. coli* O157:H7 and *Salmonella* spp. in strawberry puree treated with HPP at 450 MPa for 2 min at 21 °C (initial sample temperature) reduced over 5.4 log CFU/g and 6.0 log CFU/g (Huang et al., 2013). Mukhopadhyay et al. (2016) also reported that log reduction for cantaloupe puree treated with HPP at 300 MPa and 400 MPa, 8 °C and reached to a maximum of 35 °C for 5 min were 2.4 and 4.5 log CFU/g, respectively, for *Salmonella* spp. and 1.6 and 3.0 log CFU/g, respectively, for *L. monocytogenes*. In addition, Shahbaz et al. (2016) showed that the population of *L.*

monocytogenes, E. coli O157:H7, and *S.* Typhimurium in commercial apple juice treated with HPP at 400 MPa for 1.0 min at 25 °C and reached to a maximum of 28 °C reduced by 2.93, 1.8, and 0.49 log CFU/ml, respectively.

2.6.2.7 Ionizing radiation

Ionizing radiation is commonly used in food processing and sterilization (IAEA, 2015a), air and water treatment (IAEA, 2007), polymer processing (IAEA, 2004), and generation of biomaterials (IAEA, 2015b). It is a type of radiation from the high-energy side of the electromagnetic spectrum. Its energy is enough to produce ions and electronically charged atoms or molecules by removing tightly bound electrons from the orbit of atoms or molecules. The sources of ionizing radiation can be gamma rays, X-rays, and accelerated electrons (electron beams) (Farkas and Mohacsi-Farkas, 2011; Roberts, 2014; Lung et al., 2015). Gamma rays from radioactive isotopes such as cobalt-60 (1.17 and 1.33 MeV) and cesium-137 (0.662 MeV) are used for radiotherapy, medical devices, and the irradiation of foods whereas the usage of the radioactive isotopes has been replaced with electron beam accelerators due to their rising prices and public concerns related to the nuclear industry (Tauxe, 2001; Miller, 2005). X-rays (maximum energy 7.5 MeV) are produced when highly accelerated electrons penetrate a thin foil of metal such as tungsten or tantalum, but this technology may be impractical due to the low generation efficiency (Miller, 2005).

Electron beam technology (maximum energy 10 MeV) is safer, highly efficient (60-80 %), and the equipment can be located it anywhere in the world (Lung et al., 2015; Han et al., 2016; Wojnarovits et al., 2018). Electron beam (e-beam) irradiation utilizes a

stream of high-energy electrons produced by a linear accelerator (Miller, 2005). This technology can be used to treat very large quantities of product in a short time due to its high dose rate (1000-3000 Gy/s) compared to gamma rays (4-13 Gy/s) (Pillai and Shayanfar, 2015).

All three types of ionizing radiation are similar in terms of radiation chemistry (Fan, 2012). When charged particles enter in matter such as water, they lose their energy by interacting with the electrons from the orbit of atoms (von Sonntag, 1987). The electron beams include the fast electrons possessing energies comparable to those of gamma and X-rays and are accordingly eligible to interact with additional electrons from atoms or molecules, forming free radicals that are very reactive and can in turn ionize other atoms and molecules, creating charged or excited atoms and molecules (Miller, 2005).

In radiation chemistry, the yield of a chemical yield, *G(X)*, is described as the quotient of the amount *n (X)* of a substance of a specified entity, *X*, produced, destroyed, or altered by radiation, by the mean energy passed on, *E*, to the irradiated matter (Wojnarovits et al., 2018). The International System of Unit (SI) of the G-value is mol/J used to indicate the number of molecules produced destroyed or changed per 100 eV of energy absorbed. The conversion to SI unit is as 1 molecule $(100\ eV)^{-1} = 1.036 \times 10^{-7}$ mol/J (von Sonntag, 1987).

$$G(X) = \frac{x(X)}{E} \qquad [2.1]$$

2.6.2.7.1 Radiolysis of water

Ionizing radiation generates plentiful secondary electrons quickly decreased to energies below 7.4 eV, which is the threshold to produce electronic transitions in liquid water (von Sonntag, 1987). Both primary charged particle and secondary electrons cause the decomposition of water, whereby a water radical cation and an electron is produced (Eq. 2.2 and Eq. 2.3). The water radical cation (Eq. 2.2) is a very strong acid and rapidly loses a proton to neighboring water molecules thereby forming hydroxyl radical (˙OH) (Eq. 2.4). Simultaneously, the electron in Eq. 2.2 becomes hydrated (e_{aq}^-) by water (Eq. 2.5). The electronically excited water in Eq. 2.3 can also break down to hydroxyl radical and hydrogen ion (H˙) (Eq. 2.6) (von Sonntag, 2006).

$$H_2O \xrightarrow{ionizing\ radiation} H_2O^{\cdot+} + e^- \quad [2.2]$$

$$H_2O \xrightarrow{ionizing\ radiation} H_2O^* \quad [2.3]$$

$$H_2O^{\cdot+} \longrightarrow {}^{\cdot}OH + H^+ \quad [2.4]$$

$$e^- + nH_2O \longrightarrow e_{aq}^- \quad [2.5]$$

$$H_2O^* \longrightarrow {}^{\cdot}OH + H^{\cdot} \quad [2.6]$$

There is always the likelihood that these free radicals interact with one another such as the reactions of ˙OH with e_{aq}^- (Eq. 2.7, with relative rate of reaction $k_7 = 3.0 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) or with H˙ (Eq. 2.8, $k_8 = 7.0 \times 10^9$ dm^3 mol^{-1} s^{-1}). Furthermore, the self-termination of two ˙OH induces the formation of H_2O_2 (Eq. 2.9, $k_9 = 1.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}), whereas H_2 is formed in reaction Eq. 2.10 and 2.11 ($k_{10} = 1.55 \times 10^{10}$ dm^3 mol^{-1} s^{-1}, $k_{11} = 1.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) (von Sonntag, 2006)

$${}^{\cdot}OH + e_{aq}^- \longrightarrow OH^- \quad [2.7]$$

$$^{\cdot}OH + H^{\cdot} \longrightarrow H_2O \quad [2.8]$$

$$2^{\cdot}OH \longrightarrow H_2O_2 \quad [2.9]$$

$$2H^{\cdot} \longrightarrow H_2 \quad [2.10]$$

$$2e_{aq}^{-} + 2H^{+} \longrightarrow H_2 \quad [2.11]$$

In the radiolysis of water, hydrated electrons (e_{aq}^{-}), hydroxyl radicals ($^{\cdot}OH$), and hydrogen ion ($H^{\cdot}$) reactive intermediates are generated in the primary processes. Hydroxyl radicals are the primary reactive intermediates in radiation induced-cell lethality of foodborne pathogens such as *Salmonella* and *E. coli* due to their damage to proteins, lipids, DNA, and RNA (von Sonntag, 2006). The summary equation of water radiolysis at 25 °C is:

$$H_2O \longrightarrow [0.28] \cdot OH + [0.27]e_{aq}^{-} + [0.06] \cdot H + [0.07]H_2O_2 + [0.27]H_3O + [0.05]H_2 \quad [2.12]$$

The quantities in the brackets are the radiation chemical yields (G-values) produced per 100 eV of energy absorbed (von Sonntag, 2006).

Oxygen (O_2) has a great effect on the course of water radiolysis and the following reaction of the primary radicals in the water (von Sonntag, 2006). In aerated solution, hydrated electrons (Eq. 2.13) and hydrogen ions (Eq. 2.14) mostly react with dissolved oxygen with rate constants of 2.1 x 10^{10} and 1.9x 10^{10} dm^3 mol^{-1} s^{-1}, respectively, and then lead to formation of $O_2^{-}/HO_2^{\cdot}$ pair. Superoxide radical anion (O_2^{-}) is the deprotonated form of hydroperoxyl radical ($HO_2^{\cdot}$) and thus has reducing properties (Wojnarovits et al., 2018). At 20 °C the water absorbs 2.8 x 10^{-4} mol dm^{-3} oxygen from the air.

$$e_{aq}^{-} + O_2 \longrightarrow O_2^{\cdot -} \quad [2.13]$$

$$H^{\cdot} + O_2 \longrightarrow HO_2^{\cdot} \quad [2.14]$$

The hydrated electron and hydrogen ion scavenging capacities (s^{-1}), *SC*, calculated from Eq. (2.15) are high, 5.3 x 10^6 s^{-1} and 3.4 x 10^6 s^{-1}, respectively, because of the high rate constants (Wojnarovits et al., 2018). Therefore, if the scavenging capacity calculated for the dissolved compound is lower than these scavenging capacities, it cannot compute with oxygen to react with hydrated electrons and hydrogen ions (Wojnarovits et al., 2018).

$$SC = k * S \quad [2.15]$$

Where *k* and *S* represent the rate constant (dm^3 mol^{-1} s^{-1}) and scavenger concentration (mol dm^{-3}), respectively.

In food irradiation application, radiolysis of water has a crucial role in changes in food components and inactivation of microorganisms. (Miller, 2005). Free radicals, such as hydroxyl radicals, hydrated electrons, and hydrogen ions may react with other food components (von, Sonntag, 1987). Hydroxyl radical is a powerful oxidizing agent whereas hydrated electron and hydrogen ions are reducing agents. Hence, both oxidation and reduction reactions during irradiation probably take place in all foods containing water (von Sonntag, 1987). For instance, these three primary radicals can decrease unsaturated bonds and the stability of conjugated rings of aromatic and heterocyclic compounds in foods as well as to reduce foodborne pathogens in foods (Fan, 2012).

In addition to food irradiation, these three primary radicals formed during radiolysis of water play an important role in wastewater treatment (Basfar, and Rehim, 2002; Melo et al., 2008; Wojnarovits and Takacs, 2017). Many studies have determined

the effectiveness of ionizing radiation, one of the Advanced Oxidation Processes (AOPs), in the disinfection of wastewater and the improvement of the water quality by reducing the chemical demand (COD), biochemical oxygen demand (BOD), and total organic carbon (TOC) all at same time, which is mainly due to hydroxyl radicals (IAEA, 2007; Oturan and Aaron, 2014). Ionizing radiation is a quite efficient process because the yield of hydroxyl radicals produced from radiolysis of water as Eq. 2.12 is higher than the other AOPs, such as catalytic, ozone, UV, Fenton, and electrochemical (Wang and Xu, 2012; Wojnarovits and Takacs, 2017).

Hydroxy radical is a powerful species and reacts with the organic and inorganic compounds non-selectively at close to diffusion-controlled rates (von Sonntag, 2006; Stefan, 2018). Therefore, water quality parameters, such as alkalinity, DOC, and inorganic ions, play a crucial impact on the ionizing radiation process performance. Alkalinity, mainly expressed as equivalents of calcium carbonate ($CaCO_3$), is a major parameter of water quality (Pangloli and Hung, 2013). It is primarily in the form of bicarbonate (HCO_3) around neutrality (pH 7.3) and a well-known scavenger of ·OH and e_{aq}^- (Buxton et al., 1988). In addition, dissolved organic matter (DOM) in the water reacts with the hydroxyl radical and may be a major scavenger in the oxidation processes or radiolysis of water due to its high rate constant for the reaction of ·OH (Westerhoff et al., 2007). For instance, the degradation rate constant of penicillin G in aqueous matrices by gamma radiation was reduced by 96% and 89% in the presence of 10 g/L peptone and glucose, respectively, due to their reactions with hydroxyl radicals formed during irradiation (Chu et al., 2018). Furthermore, inorganic ions such as chloride and bromide

ions are often present in water and react with hydroxyl radicals (Wang and Chu, 2016; Wojnarovits and Takacs, 2017). Therefore, the effect of these water characteristics should be taken into consideration to develop effective treatments for decontamination of food products.

2.6.2.7.2 Food irradiation

Food irradiation has been approved as a safe and effective food processing technology by international organizations such as Food and Agriculture Organization (FAO), the World Health Organization (WHO), the International Atomic Energy Agency (IAEA), the United States Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), and Codex Alimentarius to treat foods with ionizing radiation to enhance the safety and extend the shelf life of foods by decreasing or eliminating microorganisms and insects and by prevention of sprouting (Pillai and Shayanfar, 2015; Kobayashi, 2018). The U.S. Food and Drug Administration (USFDA) approved this technology for use on fruits and vegetables at a maximum level of 1.0 kGy (Moosekian et al., 2012). The primary advantages of this technology are the very low energy requirements and the decreased heating of the food (Meireles et al., 2016).

The International Atomic Energy Agency (IAEA) recently evaluated the development of irradiated foods for immune-compromised patients and other potential target groups with microbiological criteria (Table 2.2) (IAEA, 2011). Nonetheless, Narvaiz (2015) reported that the hygienic qualities of some ready-to-eat salads using irradiation were achieved at 1.2 kGy for chicory and mixed salad, 1.4 kGy for organic rucula, and 2.0 kGy for soy and alfalfa sprouts. In addition, most medium (1-10 kGy) –

Table 2.2. Microbiological criteria for immune-compromised patients and other potential targets groups (IAEA, 2011).

Microorganism	**Limitation**
Aerobic Plate Counts	< 500 cfu/g
Listeria spp.	not detected in 25 g
Salmonella spp.	not detected in 25 g
Yeast and Mold	< 10 cfu/g
Total coliforms	< 10 cfu/g
Staphylococcus aureus	< 10 cfu/g
Aerobic spore count	< 10 cfu/g
Anaerobic spore count	< 10 cfu/g

and high (more than 10 kGy) -level doses are not suitable for fresh produce since their quality can deteriorate through changes in appearance, flavor, and texture (Mahmoud et al., 2010; Ramos et al., 2016).

Achieving a uniform dose throughout fruits and vegetables treatment is very difficult since these food items have irregular shapes (Kim et al., 2006). Thus, some parts of the product receive a higher dose that leads to changes in firmness, aroma, color, or taste (Mahrouz et al., 2004). Therefore, one of the greatest needs in the application of irradiation treatment to fresh produce is the reduction of the dose uniformity ratio (DUR; Hallman, 2017), described as the ratio of the maximum dose (D_{max}) divided by minimum dose (D_{min}). The reduction of DUR can be achieved by various ways (Kim et al., 2006; Hallman, 2017): (i) reducing dimensions of the produce; (ii) rotating the produce exposed to the source; and (iii) simply reducing the minimum dose requirement.

2.6.2.7.2.1 Dose uniformity

The dose distribution within an irradiated fresh produce can be determined by dose mapping or by Monte Carlo calculations (Kim et al., 2007). The absorbed dose, *D*, is defined as the mean energy, *dE*, carried to an incremental quantity of matter, divided by the mass of that matter, *dM* (Miller, 2005):

$$D = \frac{dE}{dM} \qquad [2.16]$$

The SI unit of absorbed dose is the Gray (Gy), defined as the energy (joule) per unit of mass (kilogram) of the medium. The absorbed dose tends to increase with increasing depth within the material to about midpoint of the electron penetration range and then it quickly falls to low doses. This reduction in dose is because of two points: (i) low kinetic

energy electrons scatter easily when the incident angle of electron decreases and (ii) the number of knock-on electrons escaping from the surface of target increases (Kim et al., 2007). The absorbed dose rate, Gy/s, is the energy absorbed by the unit mass in unit time. The penetration depth (R) of the ionizing radiation is defined as the depth at which extrapolation of the tail of the dose-depth curve meets the X axis (Figure 2.2) (Miller, 2005).

Ionizing radiation could be used in combination with an aqueous chemical solution in fresh produce washing water to achieve significant inactivation of the total microbial load including pathogenic microorganisms as well as to reduce the dose applied the product (Doona et al., 2015). In addition, this strategy might ensure uniform dose distribution throughout the entire fruits and vegetables treatment because they contain 80-95 % water, so their densities are very similar to water (Sweat, 1974; Cherfi et al., 2014).

2.6.2.7.2.2 Mechanism of microorganism inactivation

The antimicrobial effects of irradiation technology may be separated into direct and indirect effects (Levanduski and Jaczynski, 2008). The direct effects are due to the non-specific collision of photons of radiation with the atoms in the molecules of the microorganisms (Tahergorabi et al., 2012). Disintegration of the key bio-molecules such as DNA, RNA, and membrane proteins occur when exposed to e-beams and microbial cells are incapable of division, which is referred to as cellular reproductive death (Cadet

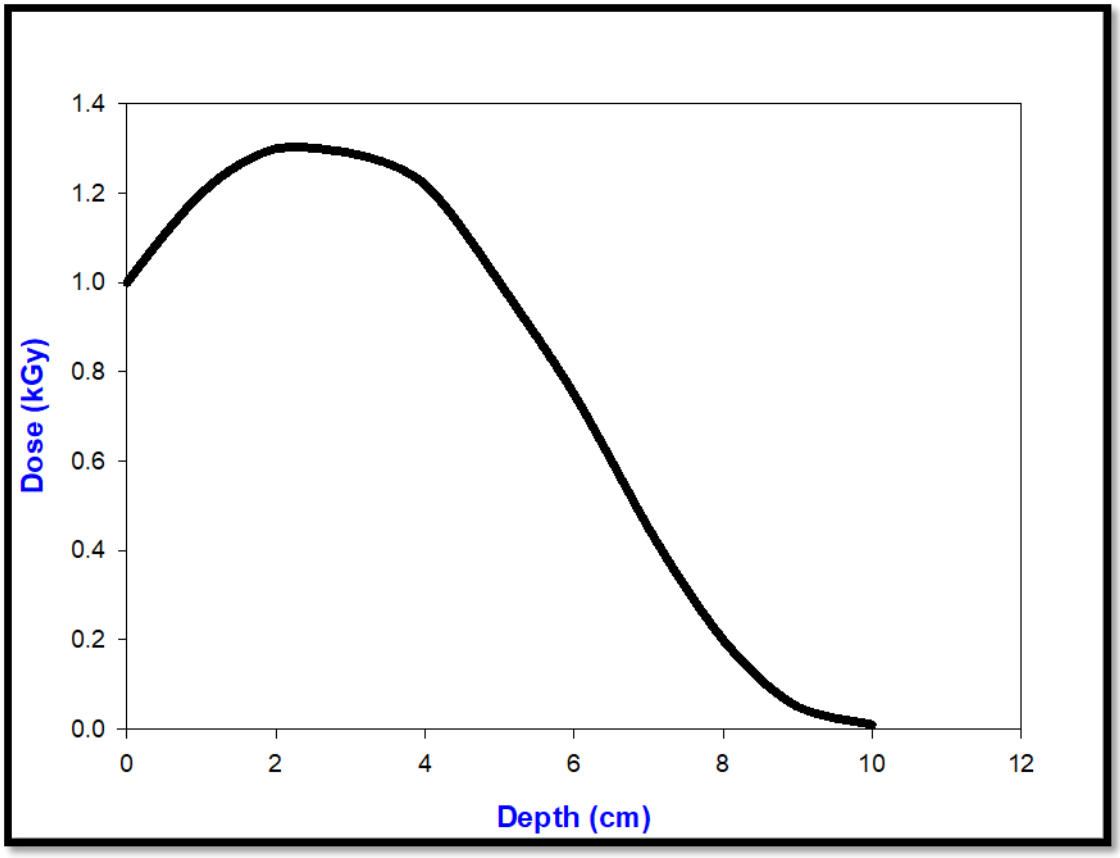

Figure 2.2. Theoretical depth-dose profile (Adapted from Miller, 2005)

et al., 1999). For direct interactions, the threshold energy was reported as 17.5 eV to induce a DNA single-strand break (Nikjoo et al., 2001).

The indirect effects are due to the free radicals created throughout water radiolysis (Li and Farid, 2016). Von Sonntag (2006) reported that approximately 99.5 % of the ionizing radiation was absorbed by the water and 0.5 % by DNA when an aqueous DNA solution including 500 mg dm^{-3} DNA was gamma irradiated, so the overwhelming contribution of free-radical damage to DNA would be caused by the free radicals formed by the radiolysis of water. The free radicals are greatly sensitive for reaction to form stable products by combining with one another or with oxygen. These oxidizing agents and free radicals are not specific only towards DNA but also cell membrane. Thus, the cellular death follows as an outcome of cellular leakage and finally complete cell lysis if the damage is adequate (Ekpanyaskun, 2009; Tahergorabi et al., 2012).

Wallace (1998) reported that ionizing radiation caused approximately 60-70% of the cellular DNA damage in mammalian cell by hydroxyl radicals that are formed from the radiolysis of water. This indirect damage was mainly generated by hydroxyl radicals in the system due to the water containing dissolved oxygen reacting with e_{aq}^- and $H^{\cdot}$ because of the high reaction rate constants (Eq. 2.13 and Eq. 2.14, respectively) and then quickly convert into superoxide radical anion ($O_2^{-}/HO_2^{\cdot}$) (Szabo et al., 2017). In addition, Samuni and Czapski (1978) found that superoxide radicals played no role in the radiodamage of *E. coli* B suspended in dilute phosphate buffer. Likewise, Kim and Thayer (1995) pointed out that superoxide radicals could not affect the radiation sensitivity of *S.* Typhimurium in phosphate buffer during gamma irradiation. From these

results, it is reinforced that hydroxyl radicals generated from radiolysis of water were mainly responsible for inactivation of microorganisms.

2.6.2.7.2.3 The effect of irradiation on pathogenic microorganisms on fresh produce

Irradiation treatment has been established as an effective method to inactivate foodborne pathogens on fresh produce (Gomes ett al., 2009; Palekar et al., 2015; Predmore et al., 2015; Meireles et al., 2016; Hallman, 2017). The penetrability and subsurface antimicrobial efficacy of irradiation treatment can play an important role decontamination pathogens internalized within the fresh produce, beyond the reach of surface sanitizers (Gomes et al., 2009). The decontamination efficacy of irradiation is affected by the target pathogen, produce type, produce condition (whole, cored, peeled or cut), and the atmosphere in which it is packed (Olaimat and Holley, 2012).

Prakash et al. (2000) showed that a dose of 1.0 kGy was needed to provide a 5-log reduction of *L. monocytogenes* in diced celery irradiated with gamma radiation. Foley et al. (2004) observed that when fresh cilantro leaves were irradiated at 1.05 kGy using gamma irradiation, a 6.70-log reduction in *E. coli* O157:H7 was achieved. In addition, Ramamurthy et al. (2004) demonstrated that gamma irradiation at 2.0 kGy was needed to completely inactivate *Listeria* and *Yersinia* (no reoccurrence) from minimally processed capsicum throughout 4 weeks of storage at 5 °C. Similarly, Shashidhar et al. (2007) indicated that irradiation at 2.0 kGy could be enough to inactivate 5 log CFU/g of *S.* Typhimurium in pineapple using gamma rays.

A study carried out by Neal et al. (2008) reported that treatment by electron beam of baby spinach at a dose 0.40 kGy resulted in a reduction in populations of *E. coli*

O157:H7 and *Salmonella* of 3.7 and 3.4 log CFU/g, respectively. In another study, determined the electron beam irradiation (up to 1.0 kGy) of baby spinach leaves resulted in ~4-log reduction of internalized *E. coli* O157:H7 (Gomes et al., 2009). Accordingly, approximately 4.2, 2.3, 3.7, and 3.6 log CFU reduction of *E. coli O157:H7, L. monocytogenes, S. enterica,* and *S. flexneri*, respectively, were achieved per tomato treated by X-ray at 0.75 kGy (Mahmoud, 2010). In the case of fresh-cut cantaloupe, Chimbombi et al. (2011) pointed out that exposure to 1.0 kGy reduced the population of *S.* Typhimurium LT2 by 1.62 to 2.65 log CFU/g for 0.5 to 30 h of growth at 23 °C using an e-beam accelerator. Palekar et al. (2015) stated that the concentration of *S.* Poona on cantaloupe irradiated with electron beams at 0.7 and 1.5 kGy decreased by 4.0-5.0 log CFU/g versus control after 21 days of storage at 5 °C.

The radiation resistance of a pathogen is usually reported as the $D_{10\text{-values}}$, which defined as the amount of radiation energy (kGy) required to inactivate 90% of specific pathogens (Fan, 2012). The $D_{10\text{-values}}$ of *E. coli* on fresh produce vary from 0.12 kGy for iceberg and green leaf lettuce (Niemira et al., 2002) to 0.47 kGy in cucumber (Lee et al., 2006). Niemira (2007) also found that the $D_{10\text{-value}}$ for *E. coli* O157:H7 cells internalized in baby spinach and romaine lettuce irradiated with gamma radiation were 0.27 kGy and 0.39 kGy, respectively, for the lower dose range (0 to 0.75 kGy). Accordingly, Rajkowski and Thayer (2000) obtained $D_{10\text{-values}}$ for *E. coli* O157:H7 of 0.34, 0.27, and 0.26 kGy on radish, alfalfa, and broccoli sprouts, respectively. In addition, Gomes et al. (2009) stated that the $D_{10\text{-values}}$ for the internalized *E. coli* on the lettuce treated by

gamma irradiation at 15 °C was as 0.34 kGy (front), 0.17 kGy (center), and 0.13 kGy (back).

The $D_{10\text{-values}}$ for *Salmonella* spp. in fresh produce varied from 0.16 to 0.54 kGy (Fan, 2012). Khattak et al. (2005) showed that the $D_{10\text{-values}}$ for *S.* Paratyphi A were 0.25 and 0.29 kGy for cucumber and cabbage, respectively, when treated by gamma irradiation at room temperature. Shashidhar et al. (2007) also determined that $D_{10\text{-value}}$ for *S.* Typhimurium in pineapple irradiated with gamma irradiation was 0.242 kGy. Another study reported that the $D_{10\text{-value}}$ of *Salmonella* spp. in baby spinach leaves irradiated with electron beam at 20 °C was 0.19 kGy (Gomes et al. 2011). More recently, Joshi et al., (2018) found that the $D_{10\text{-value}}$ of *S.* Poona inoculated on cucumber slices irradiated with electron beam irradiation was 0.38 kGy.

The radiation sensitivity of *Listeria* spp. also varies from 0.16 to 0.55 kGy for fresh produce (Fan, 2012). The average $D_{10\text{-values}}$ for a five-strain cocktail of *L. monocytogenes* in broccoli, mung bean sprouts, cabbage, and tomato irradiated with a cobalt-60 gamma source were 0.20, 0.22, 0.19, and 0.24 kGy, respectively, (Bari et al., 2005). Similarly, Gomes et al. (2011) calculated the $D_{10\text{-values}}$ for *Listeria* spp. inoculated in baby spinach leaves irradiated by electron beam at 23 °C and -5 °C were 0.21 and 0.28 kGy, respectively. Likewise, Rezende et al. (2014) determined that the average of $D_{10\text{-value}}$ was 0.21 kGy for *L. monocytogenes* in irradiated spinach by gamma irradiation.

More specifically, the radiation sensitivity of three pathogens on tomatoes have been evaluated in several studies (Prakash et al., 2007; Mahmoud, 2010; Guerreiro et al., 2016). The $D_{10\text{-values}}$ for *S.* Hartford, *S.* Montevideo, and a cocktail of *Salmonella*

enterica serovars (Poona, Hartford, Gaminara, Michigan, and Montevideo) on diced tomatoes treated with e-beam irradiation were 0.39, 0.26, and 0.32 kGy (Prakash et al., 2007). Recently, Mahmoud (2010) reported that the $D_{10\text{-values}}$ for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* inoculated on the surface of Roma tomatoes treated with X-ray at 22 °C were 0.39, 0.56, and 0.66 kGy, respectively. More recently, Guerreiro et al. (2016) obtained that the $D_{10\text{-values}}$ of 0.30 and 0.71 kGy for *S.* Typhimurium and *E. coli*, respectively, on cherry tomatoes treated with gamma irradiation at room temperature.

There are many differences in the radiation sensitivity of these three pathogens among the studies due to intrinsic and extrinsic factors (Fan, 2012; Pillai and Shayanfar, 2018). The intrinsic factors that affect the radiation sensitivity of microorganisms contain differences between species and strain of the organism (Sherry et al., 2004). The radiation sensitivity of microorganism is assumed to be inversely proportional to the size and complexity of the organism (von Sonntag, 2006; DiCaprio et al., 2016). Therefore, viruses have higher resistance to ionizing radiation than bacteria and fungi because of their very small genomes (von Sonntag, 2006). The vegetative forms of bacteria cells have lower D10-values compared to spore forms of their cells (Pinela and Ferreira, 2017). The radiation sensitivity is also affected by the growth conditions, stage of growth, the chemical and physical structure, and the number of cells (Fan, 2012).

The extrinsic factors that may affect the radiation response of microorganisms contain the irradiation temperature, presence of oxygen, and different maturity and cultivar of the produce being inoculated (Moreira et al., 2012; Pillai and Shayanfar,

2018). The radiation sensitivity of the organism increases at high temperatures, whereas it decreases at low temperatures due to the restriction of the diffusion of radicals in the frozen state (Fan, 2012). In addition, the radiation resistance of the pathogens decreases with the increased concentration of oxygen in medium (Gomes et al., 2011; Moreira et al., 2012; Karagoz et al., 2014). Furthermore, the availability of ions such as chloride ions and protective components including proteins and alcohols in medium scavenge hydroxyl radicals and decreases the radiation sensitivity of the organism (Wolcott et al., 1994; von Sonntag, 2006). For instance, Fan et al. (2005) showed that the radiation sensitivity of *L. monocytogenes* was lower in solution of calcium ascorbate compared to than in Butterfield`s phosphate buffer (BPB).

2.6.2.7.2.4 The effect of irradiation on the quality and shelf-life of fresh produce

The physiological and enzymatic activities in response to environments causes changes in quality of produce because of irradiation or storage (Fan, 2012). The effect of irradiation treatment on each quality parameters, including appearance, texture, flavor, and nutritional values, of fresh produce has been evaluated (Rico et al., 2007; Kong et al., 2014; Pinela and Ferreira, 2017). Castell-Perez et al. (2004) showed that exposure of whole and fresh-cut packaged cantaloupe to electron beam irradiation doses up to 1.0 and 1.5 kGy, respectively, did not affect ($P > 0.05$) the quality attributes of these fruits after 12 days at 10 °C. The authors also reported that carotene content of both, whole and fresh-cut cantaloupe, slightly increased when the dose increased. Moreno et al. (2006) found that irradiation of mango fruits treated by electron beam irradiation up to 1.0 kGy maintained the fruits, physical, textural, and microstructural quality attributes

and extended their shelf-life up to 21 days at 12 °C and 62.7% RH. Similarly, Gomes et al. (2008) concluded that e-beam irradiation maintained the overall quality of ready-to-eat spinach leaves irradiated up to 1.0 kGy stored 4 °C for 15 days. Recently, Yurttas et al. (2014) observed that e-beam irradiation at 1.0 kGy combined with vacuum impregnation with 2% (w/w) of ascorbic acid and 1% (w/w) of calcium lactate helped retain the whiteness and firmness of sliced mushrooms stored at 4 °C for 15 days. More recently, Tong et al. (2018) showed that the combination of vacuum impregnation and e-beam irradiation helped to maintain the quality attributes (moisture content, texture, color, total soluble solids, pH, total titratable acidity, and total phenols) of highbush blueberries impregnated with 4% (w/w) calcium lactate and irradiated up to 2.0 kGy after 14 days of storage at 4 °C.

Guerreiro et al. (2016) evaluated the antioxidant activity and quality properties such as texture, total soluble solids (TSS), and pH of cherry tomatoes irradiated with gamma source at 1.3, 3.2, and 5.7 kGy. The phenolic content corresponding to cherry tomatoes irradiated at 3.2 kGy using gamma source was significantly ($P < 0.05$) higher than that for the other irradiated and non-irradiated samples. The firmness of irradiated group of cherry tomatoes was also lower than that of non-irradiated samples and this difference was significant ($P < 0.05$) for the 5.7 kGy irradiated cherry tomatoes. In addition, an increasing tendency of TSS values for the radiation doses of 3.2 and 5.7 kGy, although only significantly different ($P < 0.05$) for the highest dose applied. Furthermore, the pH values of cherry tomatoes decreased significantly ($P < 0.05$) with irradiation at the highest dose. Similarly, significant differences ($P < 0.05$) in pH value

of tomato cubes and stem scars treated with e-beam irradiation at 0.7 and 0.95 kGy and stored for up to 15 days at 4 °C occurred after 9 days of storage (Schmidt et al., 2006).

Many studies have indicated that low-dose irradiation significantly extended shelf-life of fresh produce by eliminating spoilage microorganisms (Jouki and Khazaei, 2014; Opara et al., 2015; Ma et al., 2017; Pinela and Ferreira, 2017). Fresh produce is usually colonized by a wide variety of microorganisms, such as bacteria, yeasts, and fungi, which affects the shelf-life of fresh produce (Prakash et al., 2002; Prakash et al., 2007; Mahmoud, 2010). Prakash et al. (2000) stated that aerobic plate counts for irradiated celery by gamma irradiation at dose of 0.5 and 1.0 kGy did not exceed 7 log CFU/g while the acidified, blanched, chlorinated, and non-treated samples surpassed aerobic microbial counts of 8 log CFU/g in 22, 19, 12, and 8 days, respectively. Palekar et al. (2015) also found that although yeast on fresh-cut cantaloupe were not reduced significantly ($P > 0.05$) by e-beam irradiation and grew slowly but steadily throughout 21 days of storage at 5 °C, the counts of lactic acid bacteria (LAB) and molds on fresh-cut cantaloupes were initially reduced with 1.5 kGy ($P < 0.05$) but then LAB recovered and grew to high numbers while molds slowly decreased in both irradiated and non-irradiated samples. Guerreiro et al. (2016) achieved a 2 log CFU/g reduction on natural microbiota on cherry tomatoes treated with gamma radiation at dose of 3.0 kGy after 14 days of storage at 4 °C.

2.6.3 Hurdle Technology

The combination of different treatments, referred to as hurdle technology, is an interesting approach with potential synergistic effects (Meireles et al., 2016; Singh and

Shalini, 2016; Khan et al., 2017a). The most common hurdles used are dependent on water activity, pH, storage temperature, modified atmosphere, and addition of preservatives (Ramos et al., 2013). The aim of hurdle technology is to improve total quality of fresh produce and reduce treatment concentrations of chemicals (Khan et al., 2017; Ngnitcho et al., 2017). In addition, several parameters including process time, water usage, the number of unit processes, and energy consumption should be taken into account when a new hurdle technology is proposed (Goodburn and Wallace, 2013). Different combinations of physical and chemical methods have been adopted by various investigators to ensure the microbial safety of many fresh produce (Singh and Shalini, 2016; Wadamori et al., 2017; Mukhopadhyay and Ukuku, 2018).

Lin et al. (2002) reported that the combination of lactic acid (1.5%) and hydrogen peroxide (1.5%) at 40°C for 15 min inactivated more than 4 log CFU of *E. coli O157:H7* and *S.* Enteritidis per lettuce leaf and about 3 log CFU of *L. monocytogenes* per lettuce leaf. In addition, Gyawali et al. (2011) showed that the combination of copper (40 ppm) with lactic acid (2000 ppm) reduced *E. coli O157:H7* on surface of lettuce and tomatoes by 3.93 and 3.39 log CFU/g, respectively. Rahman et al. (2011) pointed out that the combination of 1% citric acid and AIEW treatment at 50 °C reduced the population of *L. monocytogenes* and *E. coli* on shredded carrots by 3.97 log CFU/g and 4.0 log CFU/g, respectively. Moreover, Pyatkovskyy et al. (2017) achieved a 3.9 log CFU/g reduction of *E. coli* O157:H7 on baby spinach treated by the combination of the initial spray application of Pro-San L (0.66% citric acid, 0.036% sodium dodecyl sulfate (SDS)) followed by vacuum cooling and ozonation under pressure of 68.9 kPa.

Ramos-Villarroel et al. (2015) showed that combining pulsed light (PL) and malic acid (MA) significantly (P <0.05) inhibited microbial growth compared to than either PL or MA alone, achieving more than 3.0 log CFU/g reductions in *L. innocua* and *E. coli* populations just after processing and more than 5 log CFU/g reductions throughout 15 days of storage for fresh-cut avocado, watermelon, and mushrooms. Ge et al. (2013) pointed out that the combination of UV-C (450 mJ/cm^2) and chemical sanitizers (chlorine and peracetic acid (PAA)) caused 1.0 and 1.49 log CFU/g reduction of internalized *Salmonella* in green onions and lettuce, respectively. Likewise, the combination of ozone treatment for 1 min and followed by UV light (7.95 mW/cm^2) for 2 min yielded more than 1 and 2 log additional reductions of *E. coli O157:H7* on blueberry calyx than UV or ozone alone, respectively (Kim and Hung, 2012). Sagong et al. (2011) used the combination of ultrasonication (US) with lactic acid (2%) to decontaminate organic lettuce for 5 min at 20°C and achieved 2.75, 2.71, and 2.50 log CFU/g reductions of *E. coli O157:H7, S.* Typhimurium, and *L. monocytogenes,* respectively. Similarly, Ngnitcho et al. (2017) reported that the use of combination of calcium oxide (CaO), slightly acidic electrolyzed water (SAEW), fumaric acid (FA), and ultrasonication (US) exhibited significant reduction (P < 0.05) for bacterial pathogens *E. coli O157:H7, Staphylococcus aureus, L. monocytogenes,* and *Salmonella* spp. on lettuce by 4.7, 4.9, 4.84, and 5.08 log CFU/g, respectively.

The combination of ionizing radiation with other decontamination methods, such as chemical sanitizers, is probably the most effective strategy to decrease the required radiation dose (Foley et al., 2002); the concentration of chemicals (Dutra et al., 2017);

the process time (the case of electron beams due to the high dose rate compared with gamma-ray and X-ray irradiation), (Song et al., 2017); the cost by reducing post-harvest food losses (IAEA, 1993; Abad et al., 2017); and the risks of foodborne diseases (Omac et al., 2017) as well as improvement in shelf life of the food products (Amit et al., 2017).

Foley et al. (2004) found that chlorination (200 ppm) plus gamma irradiation (1.05 kGy) reduced the concentration of *E. coli O157:H7* on cilantro by ~7 log CFU/g but this method uses too high dose and concentration of chlorine. Similarly, Kim et al. (2011) indicated that the largest synergistic values with the combined chlorine (100 ppm), ionizing radiation (2.0 kGy), and vitamin B_1 (1000 ppm) for natural microflora in oysters and short-necked clams were 2.61 and 2.68 log CFU/g, respectively. Kim et al (2012) also demonstrated that the largest synergistic value for natural microflora in mussel and squid were 2.74 and 2.77 log CFU/g, respectively, when the combined treatment was 150 ppm of chlorine and 1 kGy of irradiation. In addition, Boumail et al. (2016) observed a synergistic effect between antimicrobial coatings and gamma irradiation. The authors treated cauliflower with gamma irradiation yielded a radiation D_{10} value of 0.25 kGy for *L. innocua* while the combination of gamma irradiation with a bioactive coating yielded a D_{10} value of 0.22 kGy. Thus, the radiation resistance of the bacterium on cauliflower decreased with the bioactive coating.

Gomes et al. (2011) found that modified atmosphere packaging (MAP) (N_2:O_2 [1:1] and 100% O_2) and e-beam irradiation have a synergistic effect on microbial decontamination. The radiation sensitivity of *Salmonella* spp. and *Listeria* spp. inoculated into baby spinach leaves increased (P<0.05) with increasing oxygen

concentration. Similarly, Karagoz et al. (2014) showed that the $D_{10\text{-values}}$ for *S.* Typhimurium in the dorsal grooves of pecans in vacuum-packed (VP), nitrogen-packed (NP), oxygen-packed (OP), and air-packed (AP) were 0.44, 0.38, 0.34, and 0.36 kGy, respectively. The authors also reported that the $D_{10\text{-values}}$ for *E. coli* spp. in the dorsal groove pecans in VP, NP, OP, and AP were 0.46, 0.40, 0.36, and 0.38 kGy, respectively. The radiation resistance of *S.* Typhimurium and *E. coli* decreased with the application of MAP including oxygen.

The combination of ionizing radiation with chemical sanitizers such as hydrogen peroxide (H_2O_2) can reduce the concentration of sanitizer needed in the process of water disinfection and maintain the quality of process water as presented in Figure 2.3 (Iqbal and Bhatti, 2015; Wojnarovits et al. 2018). Arai et al. (1986) determined that the combination of gamma radiation with ozone was more effective in removing humic acid in water than ozone or gamma radiation alone. Additionally, Drzewicz et al. (2004) found that a dose of 10 kGy was required for complete 2,4-dichlorophenoxyacetic acid degradation in tap water, and a 90% conversion of organic chlorine into chloride ions when applying e-beam treatment, whereas the same result was achieved with a dose of 2.7 kGy with the addition of 1.33 mmol dm^{-3} ozone (O_3). In addition, the simultaneous combination of e-beam with O_3 resulted in an additive effect with regard to degradation and in a synergistic effect with regard to the total organic carbon (TOC) and dissolved organic carbon (DOC) reduction. Duarte et al. (2004) found that the combination of ionizing radiation and titanium dioxide (TiO_2) enhanced the efficiency of the process used to treat industrial effluent.

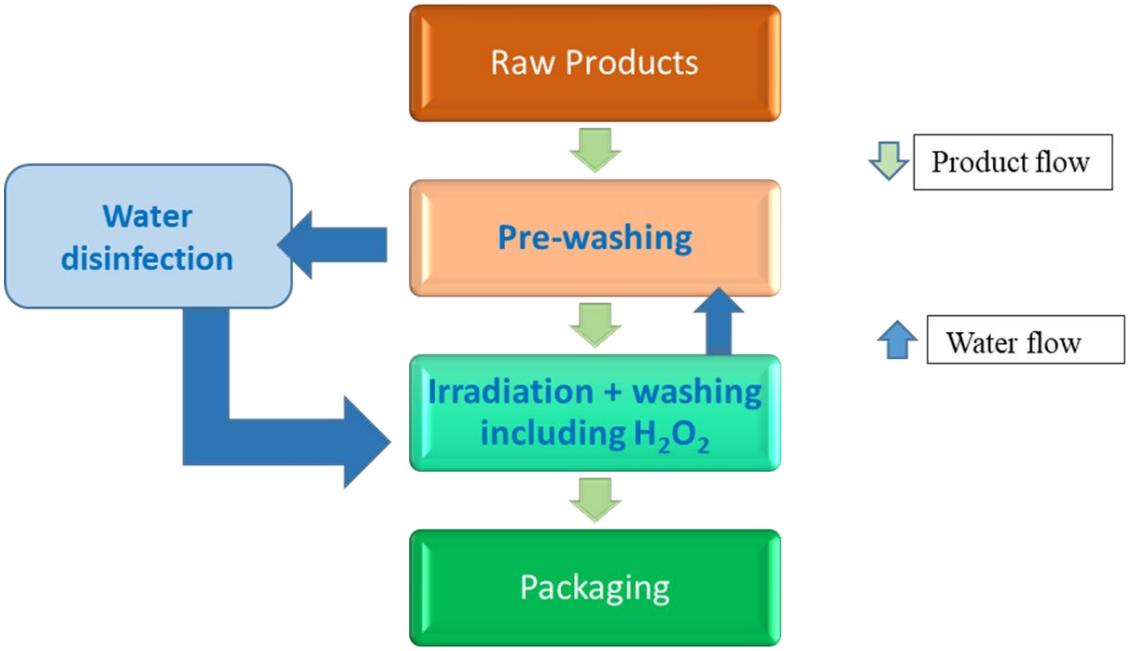

Figure 2.3. Combination of ionizing radiation with chemical sanitizers system proposed as an alternative for the traditional decontamination system for fresh produce.

Several studies have reported that the ·OH radical formation increased in the presence of H_2O_2 in aqueous solutions (Hirakawa et al., 2007; Zhang and Nosaka, 2014; Nakabayashi and Nosaka, 2015). Emmi et al. (2012) reported that when the concentration of H_2O_2 was between 5 to 10 mM, the yield of ·OH radicals in the radiolysis of an aqueous/H_2O_2 system for water treatment increased but exceeding these H_2O_2 concentrations reduced the ·OH yield. Li et al. (2011) indicated that the combination of vaporized H_2O_2 (2.52%) with UV light resulted formation of hydroxyl radicals reduced the concentration of murine norovirus 1 (MNV-1) on iceberg lettuce by approximately 2 log units within 5 min of exposure and consumption of water and disinfectant. Recently, Iqbal and Bhatti (2015) found that gamma radiation/H_2O_2 treatment was efficient for removal of nonylphenol polyethoxylates (NPEO) from wastewater treatment because of the formation of hydroxyl radicals. Similarly, Chu et al. (2018) determined that the TOC removal efficiency was increased from 21.7% by gamma irradiation alone at 10 kGy to 26.7% with 1.0 mM H_2O_2 addition due to the increased amount of hydroxyl radicals formed. More recently, Wang et al. (2019) stated that throughout gamma irradiation, H_2O_2 molecule could produce two hydroxyl radicals. These findings confirm that H_2O_2 could be used to increase the yield of ·OH radicals during the radiolysis of water exposed to electron beam irradiation. Thus, addition of H_2O_2 during electron beam irradiation of fresh produce in wash water should be evaluated since it should enhance the decontamination effect by accelerating the production of hydroxyl radicals without any negative effect on the quality of produce.

2.7 Predictive microbiology and microbial death kinetics

In predictive microbiology, mathematical models are used to describe the behavior of microorganisms given certain or dynamic environmental conditions (Peleg, 2006). These models can be used to predict the growth, survival, and inactivation of pathogenic and spoilage microorganisms in food products. In addition, the implementation of these predictive models helps to improve the control of food safety and spoilage by quantifying the effect of application of any technology in food process. Furthermore, predictive models may be an essential tool for risk control in the optimization of food engineering processes (Peleg, 2006).

Predictive models regarding microbial kinetics (growth or survival) can be divided based on the studied microbial process and the variables considered (Perez-Rodriguez and Valero, 2013). The most used categorization for predictive models is as follows (Perez-Rodriguez and Valero, 2013): (1) Primary models which define how the number of microbial cells develops as a function of time or dose given certain constant environmental factors such as temperature and chemical agent; (2) Secondary models which display how the parameters from the primary models alter with changing environmental conditions; (3) Tertiary models that define user-friendly software packages such as ComBase and Pathogen Modeling Program (PMP), where primary and secondary models are combined.

There are two steps for classic kinetic modeling in predictive microbiology (Van Derlinden et al., 2012). First, primary models are established and then, the effect of altering environmental conditions, such as temperature or chemical agent, on the parameters of these primary models is summed up in the secondary models. Thus, the

combination of primary and secondary models can be used for modeling of cell density in a dynamic environment (Van Derlinden et al., 2012).

Survivor curves are generally defined by plotting to logarithm of the number of microorganisms surviving against the size of treatment such as time, dose, or concentration (McKellar and Lu, 2004). Survivor curves of microorganisms treated with irradiation may be linear, convex, or concave as those reported for heat inactivation (see next section) (Casolari, 2018). Therefore, a function for heat inactivation may define these types of survivor curves (Casolari, 2018). The lethal effects of radiation are expected to be primarily through the indirect effect due to the formation of free radicals from radiolysis of water (von Sonntag, 1987). Microbial resistance to irradiation can be affected by substances in the medium such as the hydroxyl radical scavengers (Kim and Thayer, 1995). Also, the higher content of -SH groups per cell increase the radiation resistance of microbial cells (Bruce et al., 1969).

2.7.1 Primary models

2.7.1.1 Bigelow model (Log-linear (LL) model)

This model was built by Bigelow and Esty (1920) to quantify microbial inactivation in the canning industry, assuming first-order kinetics or log-linear model, and the principles of this model still form the basis of irradiation processes currently used by the food industry (Peleg and Cole, 1998). According to this model, the inactivation of microorganism results from the inactivation of a single molecule or site per cell, and thus the inactivation rate is expected to be proportional to the number of organisms remaining alive and follows first-order kinetics (Peleg, 2006).

Let N_o be the initial number of microorganisms (CFU/ml) and N their number (CFU/ml) after dose, D (kGy). According to the first-order kinetics model, the inactivation rate dN/dD is;

$$\frac{dN}{dD} = -kN \quad [2.17]$$

Where k is an exponential rate constant with unit of $1/D$.

Integrating Eq. 2.16 yields:

$$N = N_o \exp(-kD) \quad [2.18]$$

If the survival ratio, S, is described as N/N_o, then:

$$\ln S = -kD \quad [2.19]$$

When expressed as $\log_{10}$, gives:

$$\log S = -k'D \quad [2.20]$$

Where $k=k'*ln\ (10)$. The well-known $D_{10\text{-value}}$ meaning dose required for a 1-log reduction is equal to $1/k'$, where k' is the slope (Figure 2.4, $\beta = 1$ (see next section)).

2.7.1.2 Weibull model

The Weibull model describes the microbial inactivation based on various distributions of resistance or sensitivity between the individuals in a microbial population. This model assumes nonlinearity of semi-logarithmic survivor curves in the inactivation process. In terms of survival curves, the cumulative function is (Perez-Rodriguez and Valero, 2013):

$$\log S = -\frac{1}{2.303}\left(\frac{D}{\alpha}\right)^{\beta} \quad [2.21]$$

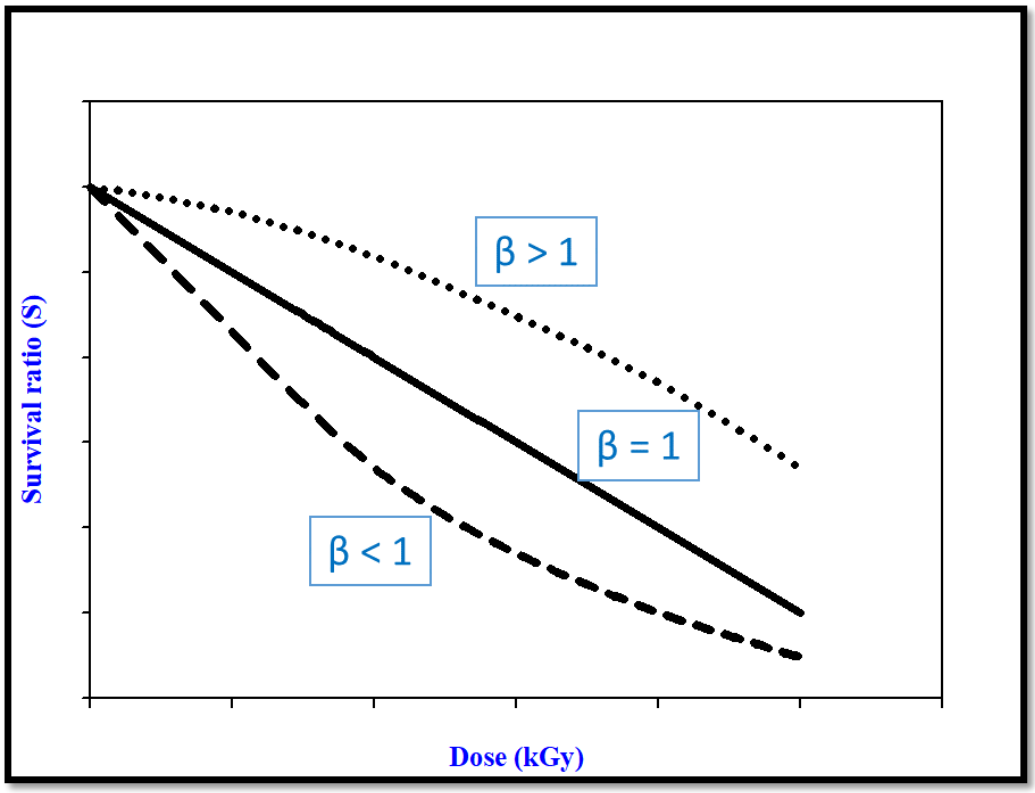

Figure 2.4. Theoretical survival curves (Adapted from Peleg, 2006)

Where α and β represent parameters related to the scale and shape of the inactivation curve, respectively. The shape parameter accounts for upward concavity of a survival curve (β < 1), a linear survival curve (β = 1), and downward concavity (β > 1) (Figure 2.4). In addition, β < 1 shows that the surviving cells have the ability to adapt to the applied stress while β > 1 shows that the surviving cells become more damaged (Perez-Rodriguez and Valero, 2013).

2.7.1.3 Log-logistic model

Log-logistic model was developed by Cole et al. (1993) to define the non-linear thermal inactivation of microorganisms (Chen and Hoover, 2003):

$$\log N = \alpha + \frac{\omega - \alpha}{1 + e^{4\sigma(\tau - \log D)/(\omega - \alpha)}} \quad [2.22]$$

Where α and ω represent the upper and lower asymptote (log CFU/ml), respectively, σ represents the maximum inactivation rate (kGy^{-1}) and τ represents the position of the maximum slope (kGy).

2.7.1.4 Log-linear plus shoulder (LLS) model

Log-linear plus shoulder (LLS) model was developed by Geeraerd et al. (2000) to model the shoulder phase of an inactivation curve based on the assumption that a critical component inside or outside the cell is destroyed by the stress conditions. The shoulder shows a period throughout which the cells can resynthesize this critical component and death occurs only when the rate of destruction exceeds the rate of synthesis (Van Derlinden et al., 2012).

$$N = N_o e^{(-k_{max} D)} \frac{(e^{k_{max}})SI}{1 + (e^{-k_{max} SI} - 1)e^{(-k_{max} D)}} \quad [2.23]$$

Herein k_{max} is the maximum inactivation rate (kGy^{-1}) and *SI* is the shoulder length or the length of the lag phase (kGy) (Figure 3.4).

This model has been used in several studies to fit microbial survival curves from different microorganisms (Geeraerd et al., 2005; Arroyo et al., 2011; Condon-Abanto et al., 2016; Kourdali et al., 2018). Geeraerd et al. (2005) compared the results of the eight models including log-linear, log-linear plus + shoulder, log-linear + tail, log-linear + shoulder + tail, Weibull, Weibull + tail, biphasic, and biphasic + shoulder models and concluded that the LLS and Weibull models described accurately the data based on sum square error (SSE) and root mean square error (RMSE) values. Similarly, Janssen et al (2007) found that either the LLS model or the Weibull model gave the best result (high R^2_{adj} and reasonably low RMSE) compared to the log-linear, biphasic, and biphasic plus shoulder models for fitting experimental data. Gomez-Lopez et al. (2010) showed that the LLS model was slightly better than the Weibull model based on R^2 and RMSE values when used to fit data regarding inactivation of aerobic mesophilic and yeast counts in a calcium-added orange juice sonicated at 89.25 µm and 10 °C up to 10 min. In addition, Gayan et al. (2012) found that the Weibull model fitted experimental data as good as the LLS model, but it did not quantify the shoulder length. Furthermore, the authors suggested the use of LLS model for fitting concave downwards curves instead of the Weibull model. Furthermore, Gayan et al. (2013) stated that the LLS model allowed to describe independently the shoulders and the log-linear section of inactivation and to directly compare results with other previously published.

2.7.1.4.1 Modeling software

The Geeraerd and Van Impe inactivation model-fitting tool (GInaFiT), a free add-in for Microsoft-Excel, was created by Geeraerd et al. (2005) to describe 10 different types of microbial survival curves: (1) classic log-linear curves (Bigelow and Esty, 1920), (2) curves exhibiting a shoulder before a log-linear decrease (Geeraerd et al., 2000), (3) curves exhibiting a tail after a log-linear decrease (Geeraerd et al., 2000), (4) survival curves exhibiting both shoulder and tailing behavior (Geeraerd et al., 2000), (5) concave curves (Mafart et al., 2002), (6) convex curves (Mafart et al., 2002), (7) convex/concave curves followed by tailing (Albert and Mafart, 2005), (8) biphasic inactivation kinetics (Corroler et al., 2006), (9) biphasic inactivation kinetics preceded by a shoulder (Cerf, 1977), and (10) curves with a double concave/convex shape (Geeraerd et al., 2005).

2.7.2 Secondary models

2.7.2.1 Square root models

Square root models are usually used to display the influence of environmental conditions such as temperature and pH on the microbial growth rate (Perez-Rodriguez and Valero, 2013). Ratkowsky et al. (1982) developed a model describing the influence of suboptimal temperatures on the maximum specific growth rate (μ_{max}):

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad [2.24]$$

Where b is a constant (1/ °C $\mu^{1/2}_{max}$), T is the temperature (°C) and T_{min} is the theoretical minimum temperature for growth (°C). Erkmen (2003) used the Eq. (2.23) to describe the relation between temperature and the inactivation rate for different carbon dioxide pressures.

These models have also been reformulated to quantify the influence of one, or a combination of, environmental conditions on the microbial inactivation rate (Buchanan, 1993; Gil et al., 2006). Amos et al. (2001) evaluated the performance of square root-type model to describe the relation between the UV dose and the microbial inactivation rate in relation with the concentration of suspended solids. In addition, Khoo et al. (2003) used this model to describe the effect of liquid temperature, pH, and the holding time on the thermal inactivation kinetics of *E. coli*. The secondary model in both studies has the following structure:

$$\sqrt{k} = b(f_1 - f_{1*})(f_2 - f_{2*}) \quad [2.25]$$

Herein f_1 and f_2 represent two factors and f_{1*} and f_{2*} represent the biological zeros points at which no effect is observed.

2.7.2.2 The Bigelow model

The Bigelow model can be used to describe the effect of the temperature or other environmental conditions including water activity and pH on the microbial inactivation rate (Lang et al., 2017).

$$k = \frac{\ln 10}{D_{ref}} \exp[\frac{\ln 10}{z}(T - T_{ref})] \quad [2.26]$$

Where D_{ref} is the decimal reduction time at a reference temperature T_{ref} (°C) and z (°C) is the change of temperature required to accomplish a 10-fold change in the D-value.

D-value (from heat inactivation) or $D_{10\text{-value}}$ (from irradiation inactivation), as originally described in the Bigelow model, have been used to evaluate the decontamination process efficiency when various primary models (in Section 2.5.1) are used (Van Derlinden et al., 2012). For inactivation curves exhibiting a shoulder length

(SI), Van Derlinden et al. (2012) described the t_{4D} which means that the time required to obtain 4-log reductions as follows:

$$t_{4D} = SI + 4D_{ref} \quad [2.27]$$

This expression may be reformulated to acquire a more general form for accomplishing y-log reductions:

$$t_{yD} = SI + yD_{ref} \quad [2.28]$$

2.7.2.3 Arrhenius-equation type models

The original Arrhenius model was evolved based on thermodynamics to define chemical reaction kinetics (Davey, 1989).

$$k = Aexp(\frac{-E_a}{RT}) \quad [2.29]$$

Where *A* is frequency factor which is constant, E_a is the inactivation energy (J/mol), *R* is the universal gas constant (8.314 J mol^{-1} K^{-1}), and *T* is the absolute temperature (K). The construction of *lnk* versus *1/T*, the slope of curve should be a straight line. However, some apparent curvature observed when the combined effect of temperature and other processing conditions such as water activity (a_w) and pH (Davey, 1989 and 1993)

This model has been reparametrized to contain additional processing conditions such as a_w and pH by Davey (1993) as follows:

$$\ln k = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 a_w + C_4 a_w^2 \quad [2.30]$$

Where C_o, C_1, C_2, C_3, and C_4 are coefficients.

CHAPTER III

THE INFLUENCE OF INOCULATION CULTURE MEDIUM ON THE RADIATION SENSITIVITY OF *SALMONELLA* TYPHIMURIUM ATCC 13311 IN AIR SATURATED AQUEOUS SOLUTIONS

3.1 Overview

This study assessed the effect of peptone water (PW), phosphate buffered saline (PBS), phosphate buffer (PB; 1, 10, and 50 mM), and the presence of hydroxyl radical scavengers, ethanol (78.9, 394.5, and 1578 mM) and polyethylene glycol (PEG; 0.0125, 0.125, and 1.875 mM), on the radiation sensitivity of *Salmonella* Typhimurium ATCC 13311 in air saturated aqueous solutions during electron beam irradiation. It was found that the radioresistance of this organism in DI water increased ($P < 0.05$) by 19.73 % and 26.53 % when PW and PBS, respectively, was dissolved into DI water.

The increased concentration of PB, 10 mM to 50 mM, in DI water also increased ($P < 0.05$) by 6.12 % and 32.65 %, respectively, the radioresistance of this pathogen. In addition, the radiation sensitivity of *S*. Typhimurium ATCC strain 13311 in DI water including 10 mM PB increased ($P < 0.05$) by 19.23 % in the presence of 150 mM NaCI. However, the radiation sensitivity of *S*. Typhimurium ATCC 13311 irradiated in DI water by e-beams was not affected ($P > 0.05$) by the addition of 1.0 mM PB.

The addition of hydroxyl radical scavengers, ethanol and PEG (0.125 mM and over) significantly decreased the radiation sensitivity of *S*. Typhimurium ATCC strain 13311 irradiated in 1.0 mM PB by e-beam irradiation. Membrane-permeable ethanol provided

better protection than non-permeable PEG. This result showed that the hydroxyl radical, especially produced intracellular, was the primary radical species formed radiolysis of water responsible for inactivating this pathogen in aqueous solutions. Furthermore, the shoulder length, SI, estimated in the presence of high concentration of hydroxyl radical scavengers (394.5 mM and over) were higher ($P < 0.05$) than that estimated for others.

The findings obtained in the present study demonstrated that preinoculation culture used for inoculum preparation in microbial studies could significantly affect the radiation sensitivity of pathogens in foods. Therefore, to minimize the protection effect of these culture from irradiation treatment, 1.0 mM PB can be used in microbial studies.

3.2 Introduction

Application of ionizing radiation including gamma-ray, X-ray, and electron beam (e-beam) is a cold technology used to improve the safety, quality, and phytosanitary standards of fresh produce (Khan et al., 2017a; Ma et al., 2017). The U.S. Food and Drug Administration (FDA) has approved the use of this technology on fruits and vegetables at a maximum dose level of 1.0 kGy except for iceberg lettuce and spinach which can be exposed to a maximum dose of 4.0 kGy for shelf-life extension (Fan et al., 2012).

The antimicrobial effects of irradiation technology may be separated into direct and indirect effects (Levanduski and Jaczynski, 2008; Li and Farid, 2016). The direct effects are due to the non-specific collision of photons of radiation energy with the atoms in the molecules of the microorganisms (Tahergorabi et al., 2012). Disintegration of the key bio-molecules such as DNA, RNA, and membrane proteins occur when

materials are exposed to e-beams and microbial cells are incapable of division, which is referred to as cellular reproductive death (Cadet et al., 1999). For direct interactions, the threshold energy was reported as 17.5 eV to induce a DNA single-strand break (Nikjoo et al., 2001). The indirect effects are related to the free radicals created throughout water radiolysis (Li and Farid, 2016). These free radicals are highly sensitive for reaction to form stable products by combining with one another, or with oxygen. These oxidizing agents and free radicals are not specific only towards DNA but also the cell membrane. Thus, the cellular death follows as an outcome of cellular leakage and finally complete cell lysis if the damage is sufficient (Tahergorabi et al., 2012). Wallace (1998) reported that ionizing radiation caused approximately 60-70% of the cellular DNA damage in mammalian cells by hydroxyl radicals that are formed from the radiolysis of water. Hence, the degree to which the radiolytic radical formed from radiolysis of water can increase the indirect effect of e-beam irradiation on microbial inactivation on fresh produce (Moreira et al., 2010).

Since the foundation of radiation chemistry, water and aqueous solutions of many compounds have drawn attention (Buxton et l., 1988; Elliot, 1989; Ershov and Gordeev, 2008). The radiolysis of water by ionizing radiation can produce considerable quantities of oxidizing (hydroxyl radical, ·OH) and reducing (hydrogen radicals, ·H, hydrated electron, e_{aq}^-,) radical species (Xu et al., 2015) as described below:

$$H_2O \xrightarrow{radiolysis} [0.28] \cdot OH + [0.27]e_{aq}^- + [0.06] \cdot H + [0.07]H_2O_2 + [0.27]H_3O + [0.05]H_2 \quad [3.1]$$

The *G* values in brackets, defined as the number of radicals, excited states or other products formed by absorption of 100 eV of energy, describe the efficiency of

conversion of water to radicals by high energy electrons. These reactive species might be involved in the initiation and propagation of free radical chain reactions with macromolecules, such as nucleic acids, lipids, and proteins, in the cell and are potentially highly damaging to the cell (Reisz et al., 2014; Riley, 1994; Ekpanyaskun, 2009).

It has been established that the contribution of direct and/or indirect DNA damage by ionizing radiation on the radiation-induced lethality of bacteria was higher compared to that of proteins, lipids, and RNA damage (Sage and Shikazono, 2017). Similarly, Sahbani et al. (2014) pointed out that hydroxyl radicals were the most damaging species affecting the plasmid [pGEM-3Zf (-)] DNA because they generated both base damage and DNA strand breaks.

Many studies have reported that alterations in DNA following reaction with hydroxyl radical were related to changes in radiation sensitivity (Achey and Duryea, 1974; Hutchinson, 1985; Alizadeh et al., 2015). For instance, Lafleur et al. (1975) found that a low concentration (0.1 mM) of t-butanol, a hydroxyl radical scavenger, protected bacteriophage ØX174 DNA irradiated in 10 mM phosphate buffer by gamma radiation. Furthermore, Ewing and Kubala (1987) demonstrated that certain hydroxyl radical scavengers reduced the radiation sensitivity of *Escherichia coli* B/r in equilibrium with air exposed to X-rays. Similarly, Kim and Thayer (1995) reported that the addition of hydroxyl radical scavengers significantly ($P < 0.05$) decreased the lethality of gamma radiation for *S.* Typhimurium in phosphate buffer in the presence of air while not in the presence of N_2 or N_2O gases.

The ·OH radicals are highly reactive for both the oxidation of inorganic and organic substances and inactivation of microorganisms present in water (Buxton et al., 1988). Both, peptone water (PW) and phosphate buffered saline (PBS), including inorganic and organic substances, are commonly used as suspended medium in many biological studies (Beuchat, 1999; Kim et al., 2000; Cook, 2003; Hilderbrandt et al., 2016). In addition, many studies found that inorganic anions such as chloride (CI^-), dihydrogen phosphate ($H_2PO^-_4$), and hydrogen phosphate ($HPO4^{2-}$) and cations such as sodium (Na^+) and potassium (K^+) were formed during radiolysis of aqueous sodium chloride (NaCI) and phosphate buffer solutions (Anbar and Thomas, 1964; Black and Hayon, 1970; Maruthamuthu and Neta, 1978). Furthermore, Namiki et al. (1961) reported that the chloride radicals formed from an interaction of hydroxyl radicals and CI^- (or NaCI molecule itself) throughout gamma irradiation increased the radiation sensitivity of *E. coli* in distillated water in various NaCI concentrations. Likewise, Wolcott et al. (1994) determined that irradiation of *Escherichia coli* and *Streptococcus lactis* in phosphate- or sulfate- including media was ineffective, but irradiation in solutions including 150 mM NaCI or 100 mM bicarbonate buffers caused immediate loss of microbial viability due to the formation of carbonate and chloride radicals.

Brustad and Wold (1976) reported that phosphate was generally considered to be radiochemically inert, but the effect of phosphate on the bacterial radiosensitivity needs to be taken into account due to the OH-induced hydrogen abstraction reactions. More recently, Moreira et al. (2012) tested the effect of suspended medium, peptone water (PW) and tryptic soy broth (TSB), on the radiation sensitivity of *S.* Typhimurium LT2

and *E. coli* strains inoculated on fresh spinach leaves (unsealed bags) and found that the radiation sensitivity of TSB-suspended bacteria was significantly ($P < 0.05$) less than that of PW-suspended bacteria. To our knowledge there have been no reports analyzing the effects of inorganic and organic compounds in these media on the radiation sensitivity of foodborne pathogens. Therefore, there is a need to further research the effect (if any) of the inoculation medium on the radiosensitivity of the pathogens for proper design of e-beam irradiation treatments of fresh produce and other food items.

The objectives of this study were (1) to evaluate the effect of (a) deionized (DI) water alone, (b) peptone water (PW), (c) phosphate buffered saline (PBS), (d) phosphate buffer (PB), and (e) the available hydroxyl radical scavengers in water, on the sensitivity of *Salmonella* Typhimurium ATCC 13311 to electron beam (e-beam) irradiation and, (2) to quantify the inactivation kinetics of *S.* Typhimurium in DI water and the other aqueous solutions.

3.3 Materials and methods

3.3.1 Bacterial culture

Salmonella enterica subsp. *enterica* serotype Typhimurium ATCC 13311 (hereafter called *S.* Typhimurium) was provided from Dr. Castillo`s Food Microbiology Laboratory (Department of Animal Science, Texas A&M University). Frozen stocks were maintained at -80°C. Prior to use, an inoculum was removed from frozen culture with a loop, streaked onto 9 mL Trypticase Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD) and incubated at 37°C for 24 h. Then, single colony isolate was obtained by streaking on Trypticase Soy Agar (TSA; Difco, Becton Dickinson, Sparks, MD) and

incubated at 36 °C for 24 h through two successive transfers on TSA. Colonies were stored on a TSA slant at 5 °C as working cultures and used within 30 days.

3.3.2 Inoculum preparation

In general, the inoculation procedure of Keskinen et al. (2009) was followed with minor modifications. The inoculum was prepared by transferring a loopful of the working stock to 9 mL TSB and incubated at 37°C for 18-24 h. After incubation, each test tube was centrifuged and washed for three consecutive times (3000 x g for 15 min) with sterile deionized (DI) water at 5°C. Each of the cell pellets obtained was resuspended in 9 mL sterilized DI water or sterilized treatment solutions. The average final concentration of *S.* Typhimurium was about 10^9 CFU/ml as checked by plate counting on TSA. Before each experiment, fresh cultures were prepared.

3.3.3 Preparation of aqueous solutions

One g dehydrated peptone water (PW, Criterion, Hardy Diagnostics, Santa Clara, CA) was dissolved in 1000 mL of DI water. Initially, 1000 mM potassium phosphate (monobasic, ACS grade) (KH_2PO_4, EMD Millipore Cooperation, Billerica, MA), 1000 mM potassium phosphate (dibasic, ACS grade) (K_2HPO_4, EMD Millipore Cooperation, Billerica, MA), and 1000 mM sodium chloride (NaCI, EMD Millipore Cooperation, Billerica, MA) was made by dissolving 136.09 g, 174.2 g, and 58.44 g, respectively, in 1000 ml DI water. For the stock solution of phosphate buffer (PB, 50 mM total phosphate at pH: 7.2), 16.8 ml of 1000 mM KH_2PO_4 and 34.2 ml of 1000 mM K_2HPO_4 were mixed in enough DI water to make volume 1000 ml. In addition, 200 ml of 50 mM PB and 150 ml of 1000 mM NaCI was mixed in 650 ml DI water for the stock solution

of phosphate buffered saline (PBS), including 10 mM phosphate buffer and 150 mM NaCI. For the stock solution of hydroxyl radical scavengers, 1.5 g Biotechnology grade (> 99.0 %) of polyethylene glycol, M_w 8,000 (PEG, Amresco Inc., Solon, OH) and 7.27 g ethanol (100 %) (Koptec, Decon Lab., King of Prussia, PA) were dissolved in 100 ml non-sterile 1 mM PB and sterile 1 mM PB, respectively. All aqueous solutions, except the ethanol solution, were sterilized by autoclave for 15 minutes at 121°C and their pH values were separately measured using a digital pH meter (FE20/EL20, Mettler Toledo™, Columbus, OH, USA). Table 1 shows the experimental design.

Other experiments were performed with two hydroxyl radical scavengers, ethanol (membrane permeable) and polyethylene glycol (PEG, membrane impermeable) at various concentrations added to water with 1 mM PB. Both ethanol and PEG have been used in biological studies (Sanner and Pihl, 1969; Michaels and Hunt, 1978; Samuni and Czapski, 1978; Ewing and Kubala, 1987) as specific hydroxyl radical scavengers that react with hydroxyl radical with a biomolecular rate constant of 1.9×10^9 $M^{-1}s^{-1}$ and 4×10^8 $M^{-1}s^{-1}$, respectively (Buxton et al., 1988).

3.3.4 Sample preparation

Non-sterile cylindrical sample vials (14 mm diameter) x 1 mm height) (Dynalon Labware, Dynalab Corp., Rochester, NY, USA) were initially sterilized using 70 % ethanol and air dried in a biological safety cabinet (Class II Type A2, Labconco Corporation, Kansas City, MO, USA) for an hour (Jadhav et al., 2013). Each sample was then prepared from 150 µl of the bacterial suspension as described in Section 3.2 and

Table 3.1 Experimental design (type of aqueous solutions tested and concentration levels)

Treatment	Concentration (mM)	pH
Deionized (DI) water		7.3[#] (0.09)*
Peptone water (PW)	10 g gelatin peptone and 5 g NaCI per liter	7.2 (0.12)
Phosphate buffered saline (PBS)	10.0 mM PB and 150 mM NaCI	7.2 (0.15)
Phosphate buffer (PB)	1.0	7.2 (0.05)
	10.0	7.2 (0.10)
	50.0	7.2 (0.10)
Ethanol	78.9.0	7.2 (0.08)
	394.5	7.2 (0.08)
	1578.0	7.2 (0.12)
Polyethylene glycol (PEG)	0.0125	7.2 (0.10)
	0.125	7.2 (0.12)
	1.875	7.2 (0.15)

[#]Values are means of three replications (n = 9)

*Standard deviation

placed in the sterilized sample vial for electron beam irradiation treatment. To check the sterility of the cylindrical sample vials, three negative control samples were prepared as 150 µl sterilized deionized water without bacterial suspension, before each experiment. Sample vials were then sealed with Parafilm (Bemis NA, Neenah, WI, USA) and placed in heat-sealed sterile Whirl-Pak® bags (Whirl-Pak, NASCO, Fort Atkinson, WI). Afterward, samples were transported to the e-beam facility in an insulated cooler with refrigerant packs. At the accelerator facility, samples were allowed to equilibrate to room temperature (~ 22 °C) for 15 min prior to irradiation.

3.3.5 Electron beam (e-beam) irradiation treatment

The irradiation tests were carried out with a 1.35 MeV Van de Graaff accelerator (High Voltage Engineering Corp, Cambridge, MA) located at the Food Safety Engineering lab in the Hobgood building at Texas A&M University. A Farmer ionization chamber (Markus® Ion Chamber, Type 23343, Radiation Design, Inc., Albertville, MN, USA) was used as a primary standard dosimeter for calibration procedure (Kim et al., 2007) and, to determine the hot spot, which is the location of maximum electrons emitted on the plate about 15 cm away from the electron gun. Then, a radiochromic film dosimeter (RF, Far West Technology Inc., Batch 1086, 42.5µm, Goleta, CA, USA) was placed at the hotspot and irradiated with the target dose. After irradiation, a digital radiochromic reader (Model FWT-92D, Far West Technology Inc., Goleta, CA, USA) was used to read the optical density of the RF. The absorbed dose in kilogray (kGy) was linearly correlated to the optical density of the RF and used for further measurement of the dose absorbed by the samples.

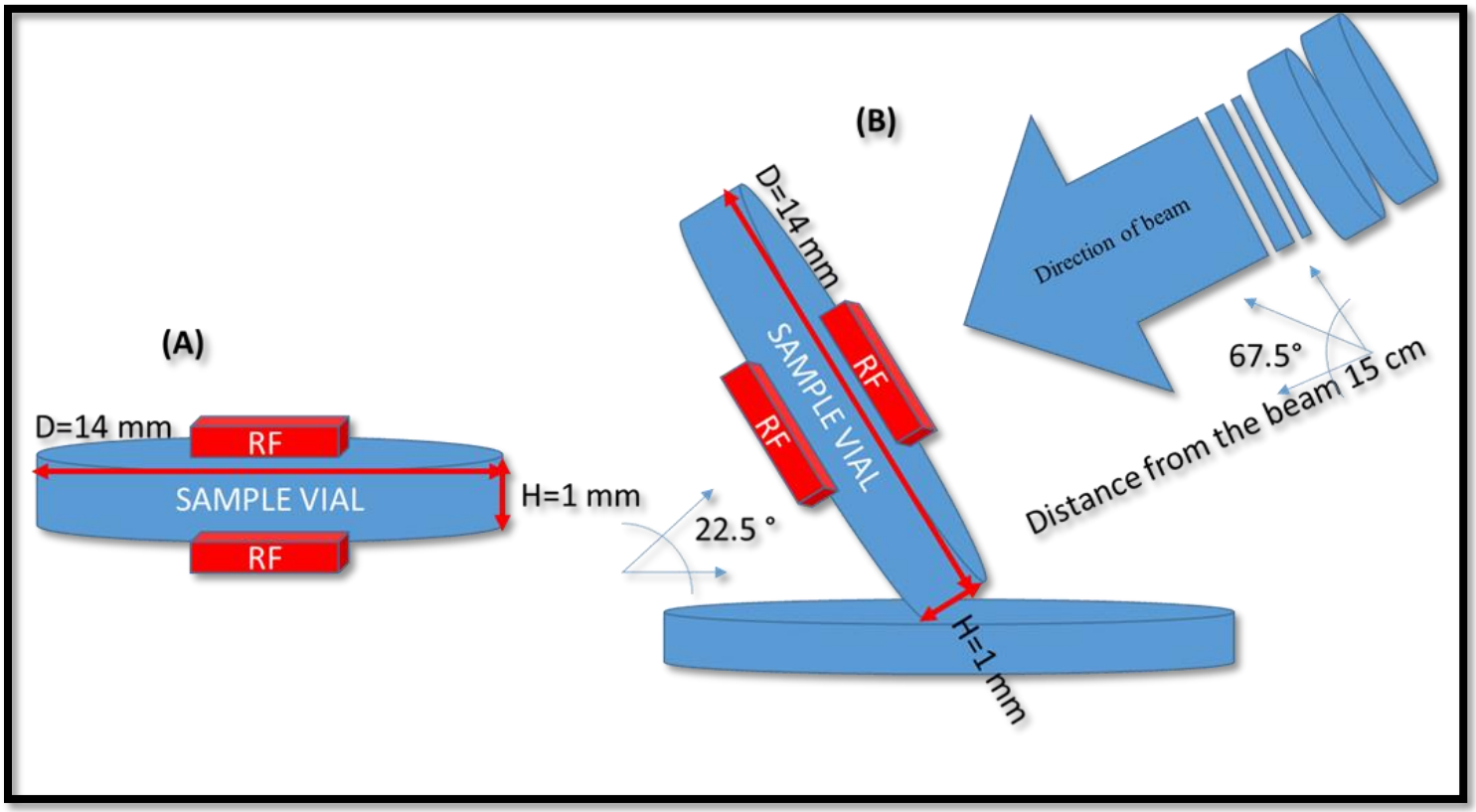

Figure 3.1 Placement of radiochromic film (RF) dosimeters on the sample vial for irradiation tests using 1.35 MeV Van de Graaff linear accelerator at room temperature (~22 °C). (A) Front view, (B) Side view

3.3.5.1 Dose mapping

Two RFs (one on the front and one on the back of the sample) were used to measure the absorbed dose for each point of the target dose (Figure 3.1, Kim et al., 2006). The dose absorbed by the RF placed on the front of the sample is defined as the entrance dose, used to calculate actual inactivation kinetics. Three independent samples were prepared by irradiating them on front-side at room temperature at the dose levels of 0.15, 0.30, 0.45, 0.60, and 0.75 kGy. This dose range was chosen based on preliminary studies which showed that when a dose of 1.0 kGy was used, the number of microorganisms surviving decreased below the quantification limit (the detection limit in the present study was 10 CFU/ml).

Moreira et al. (2012) found that the dose uniformity ratio (DUR), which is the ratio of maximum to minimum dose, D_{max}/D_{min}, affected the D_{10} values when irradiating fresh produce. Therefore, the authors used polyethylene (PE) sheets (25 mm W (Width) x 25 mm L (Length) x 0.057 mm T (Thickness)), which have composition similar to aqueous solutions (C, H, and O) and density (0.925 g/cm^3), to determine the dose distribution in the samples (Kim et al., 2006). The rationale for this procedure was that the sample vial dimensions were not suitable for placing the RF on it to measure the absorbed dose at different depths. A similar approach was followed in this study where the RFs were located on the top and bottom of 36 sheets as well as between the sheets (Figure 3.2). Thus, the absorbed dose at a particular depth was measured and the depth was calculated in terms of the areal density, A_d, as:

$$A_d = \rho * d \qquad [3.2]$$

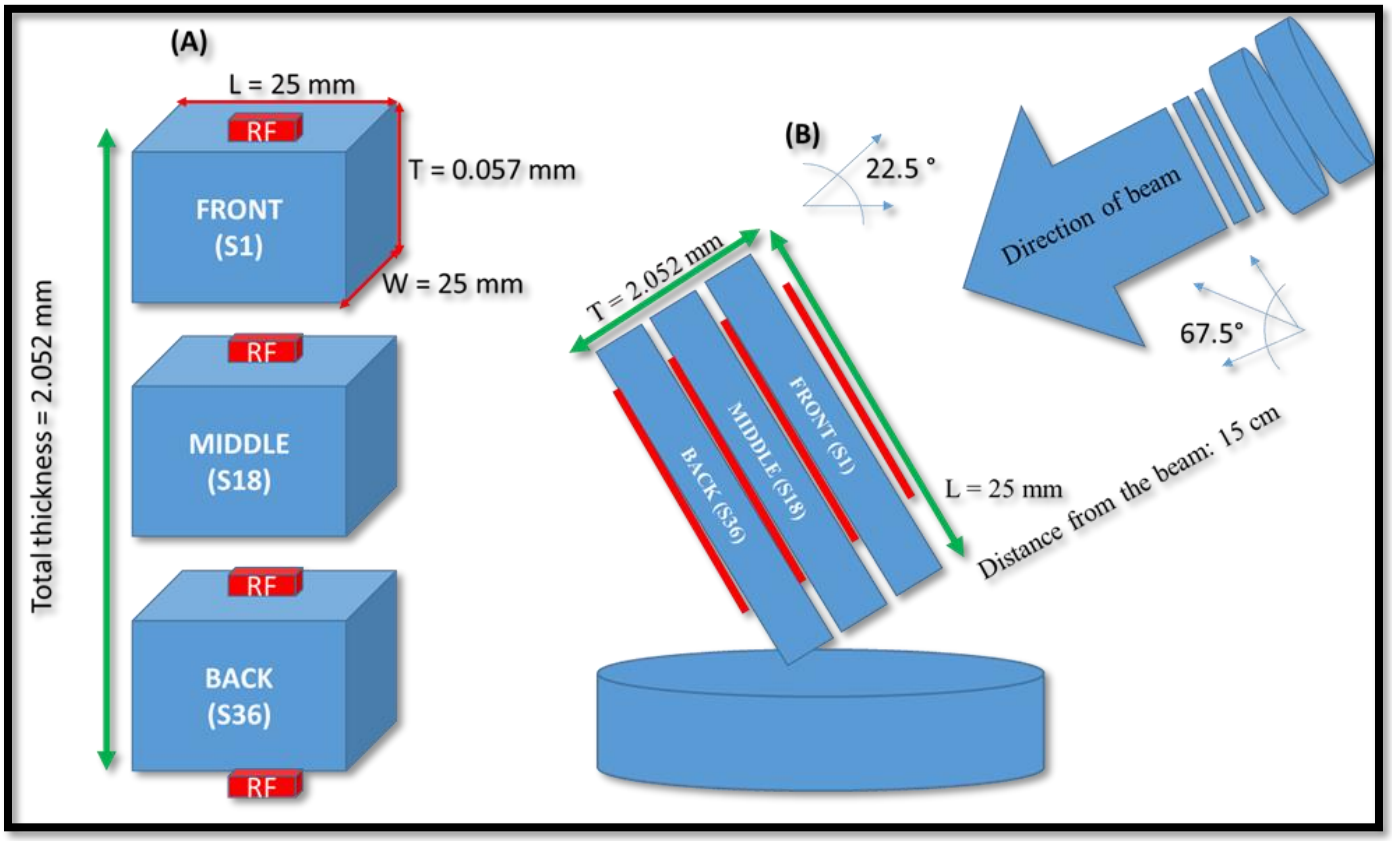

Figure 3.2 Polyethylene sheet system dimensions (A) and placement for evaluation of dose distribution per length during electron beam irradiation (B) (S1, S18, and S36 are the front layer, middle layer, and back layer, respectively, and RF is radiochromic film

where, ρ and d are the density of PE and the physical depth of the RF (Miller, 2005). Then, a plot of the absorbed dose versus areal density was developed for a target dose of 1.0 kGy (Figure 3.3). The DUR was calculated as 1.28 with the maximum and minimum dose being 1.010 and 1.296 kGy, respectively, based on dose distribution on the areal density (Eq. (2)) (Figure 3.3). This DUR value should be close to the value of 1.0 to get uniform dose distribution in small samples. However, higher values of DUR are more realistic in practical applications (IAEA, 2002).

3.3.6 Bacterial enumeration

The number of surviving *S*. Typhimurium ATCC strain 13311 on each irradiated sample was enumerated. Samples not exposed to the electron beam treatment served as controls of the initial microbial load. Samples of 0.1 ml from the original samples and 0.1 ml from serial dilution in 0.1 % of PW were plated in duplicate on TSA incubated at 37°C for 24-48 h. After incubation, visible colonies were enumerated with the use of a magnifier counter (detection limit was 10 CFU/ml). For quality purposes, dilutions with less than 10 colonies (average of 2 plates) were not considered in the calculations (Sutton, 2011).

3.3.7 Microbial inactivation kinetics

3.3.7.1 Primary models

The GlnaFiT inactivation model-fitting tool (Geeraerd et al., 2005) was used to develop the microbial survival curves (CFU/ml vs. dose) for *S.* Typhimurium ATCC 13311 and calculate the inactivation model parameters for this pathogenic strain.

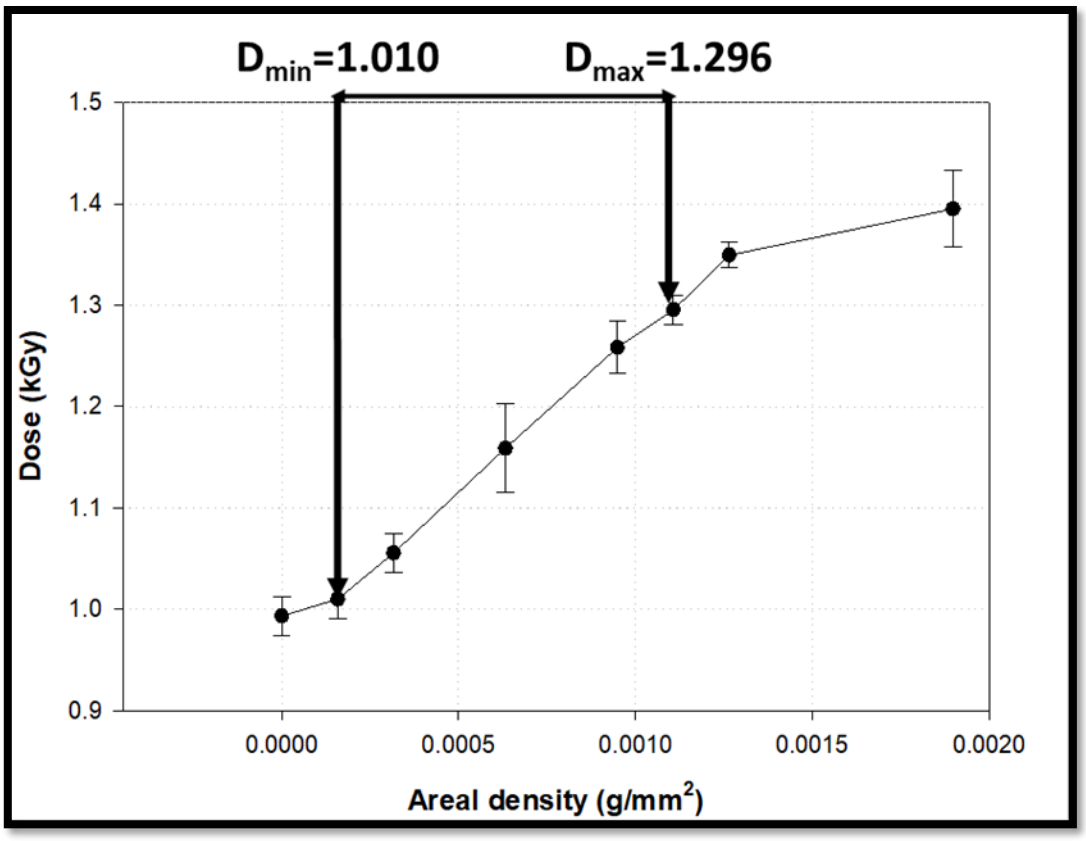

Figure 3.3 Dose distribution based on the areal density of polyethylene sheets

Two inactivation models were tested. The **log-linear model (LL**) is the simplest method to characterize microbial inactivation kinetics (Bevilacqua et al. 2015). Hence, survival curves were constructed by plotting the measured CFU/ml against radiation dose (kGy) on a semi-log graph and data fitted to the log-linear model:

$$N_D = N_0 - k * D \quad [3.3]$$

Where, N_o is the initial number of undamaged cells (CFU/ml), N_D is the number of remaining cells (CFU/ml) after exposure to dose, *D*, (kGy), and *k* is the rate constant (1/kGy). The number of surviving cells reduces exponentially and that is way it is called a "one-hit process" (Quintero-Ramos et al., 2004):

$$N_D = N_0 - e^{-kD} \quad [3.4]$$

Then, the well-known D_{10} value, defined as the dose required to inactivate 90% of the viable microorganism (Peleg and Cole, 1998), was calculated:

$$D_{10} = \frac{\ln(10)}{k} \quad [3.5]$$

The log-linear model assumes that a hit was scored when an adequately energy deposit took place within a target structure, which is the DNA in the bacterial cell (Alper, 1987; Desouky et al., 2015). On the other hand, Geeraerd et al. (2000) stated that the presence of a shoulder in a death kinetic curve could be observed due to the protective effect of the medium or some components including biological macromolecules on/ in cells and failing to resynthesize a critical component for cells affecting the magnitude of the rate constant. Several studies have reported the presence of this shoulder for bacterial inactivation by irradiation (Manas and Pagan, 2005; Chimbombi et al., 2011; Bermudez-Aguirre and Corradini, 2012). Thus, the **shoulder**

plus log-linear model (LLS) (Geeraerd et al., 2000) was also used in this study to calculate inactivation resistance parameters using the equation below

$$N_D = N_o e^{-k*SI}\left(\frac{e^{-k*SI}}{1+(e^{-k*SI}-1)e^{-k*D}}\right) \quad [3.6]$$

Where, SI is the shoulder length (kGy) presented on Figure 3.4.

For comparison purposes, the parameter '*5D*', defined as the dose required to inactivate 99.999% of the microbial population, was used in the process design criteria as recommended for irradiation of fresh produce by King and Moorman (2017). The average D_{10} value, $D_{10\text{-}average}$, was calculated using the relationship below (van Asselt and Zwietering, 2006):

$$D_{10-average} = [SI + (5 * D_{10})]/5 \quad [3.7]$$

3.3.7.2 Secondary models

Various concentration of PB, ethanol, and PEG were used to evaluate their effect on the radiation sensitivity of *S*. Typhimurium ATCC strain 13311in aqueous solutions. Therefore, the Davey (linear Arrhenius) model (Davey, 1993) was used to compare the impact of the concentration of PB, ethanol, and PEG on the inactivation rate constant, *k(D)*, as a function of dose calculated from Eq. (6) as:

$$\ln\left(\frac{1}{k(D)}\right) = A_0 + A_1 C + A_2 C^2 \quad [3.8]$$

Where, A_0, A_1, and A_2 are regression parameters and *C* is the concentration of solute (mM).

3.3.7.3 Kinetic inactivation model evaluation

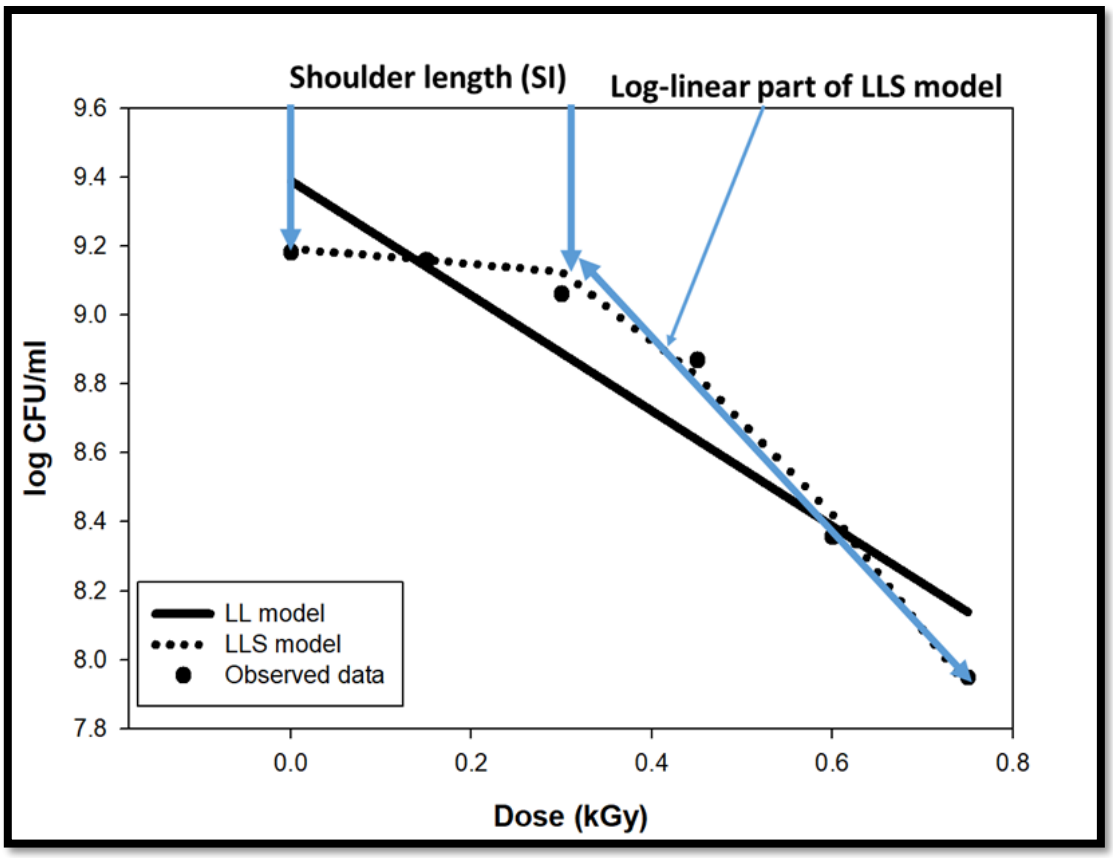

Figure 3.4 Theoretical survival curve for microorganisms fitted by the log-linear (LL) and log-linear plus shoulder (LLS) models

The suitability of the two inactivation models was determined by comparing the RMSE (root mean squared error) and coefficient of determination (R^2). The model with the smallest RMSE and highest R^2 values was considered the best fit for the respective survival curve (Geeraerd et al., 2005). The log-linear (LL) model is a two-parameter model while the log-linear plus shoulder (LLS) model is a three-parameter model, so the LLS model is expected to almost consistently fit better than the LL model. Therefore, the corrected Akaike information criterion (AICc) (Burnham and Anderson, 1998) was used to compare the models since AICc takes into account sample size and number parameters in a model. Hence, the model having the lowest AICc value is considered the best models:

$$AICc = n * \ln(\frac{SSE}{n}) + 2(p+1) + \frac{2*(p+1)*(p+2)}{n-p-2} \qquad [3.9]$$

Where, *n* is the number of observations; *SSE* is the sum of squares in the model; and *p* is the number of parameters in the model. Additionally, residuals plots and observed and prediction value plots were used to compare the log-linear (LL) and log-linear plus shoulder (LLS) models (Serment-Moreno et al., 2015).

To validate the secondary models, the accuracy, A_f, and bias, B_f, factors as presented by Eq. (3.10) and Eq. (3.11), respectively, were calculated to (Baranyi et al., 1999). The A_f and B_f provide an indication of the average deviation between the observations and predictions and structural deviations of a model, respectively (Omac et al., 2018). When the A_f and B_f are equal to 1, it represents the perfect agreement between experimental observations and model predictions (Omac et al., 2018):

$$A_f = 10^{\frac{\sum\left|\log\left(\frac{P}{O}\right)\right|}{n}} \quad [3.10]$$

$$B_f = 10^{\frac{\sum\log\left(\frac{P}{O}\right)}{n}} \quad [3.11]$$

Where, *P* and *O* are the predicted and observed values, respectively.

Statistical analysis was performed using the SPSS (version 20.0 for windows, 2011). Each parameter calculated from primary models was determined for each treatment and analyzed by an analysis of variance (ANOVA) using Tukey`s multiple range tests. Statistical significance was determined at the $P<0.05$ levels.

3.3.8 Experimental design

Six different sets of experiments were conducted (Table 3.1). DI water and aqueous solutions including PW, PBS, PB, ethanol, and PEG were used with the e-beam irradiation treatment to determine their impact on radiation sensitivity of the pathogen. The effect of each compounds on the radiation sensitivity of *S.* Typhimurium ATCC 13311 in DI water or aqueous solutions was evaluated using the primary models mentioned in Section 3.3.7.1.

Three samples were prepared for each point of target dose (0.15, 0.30, 0.45, 0.60, and 0.75 kGy) for each solution before the e-beam irradiation treatment. Three replications were done for each experiment.

3.4 Results and discussion

3.4.1 Comparison of primary kinetic inactivation models

The log-linear (LL; Eq. (3.3)) and log-linear plus shoulder (LLS; (Eq. (3.6)) models fitted the experimental data reasonably well, although neither model consistently

produced the best fit to all the survival curves (based on the goodness of fit indices). In terms of the RMSE values, the LL model only predicted better 3 (25 %) out of 12 survival curves, whereas the LLS model did better in 3/4 (75 %) of the curves, suggesting that the LLS model is a better fit because it includes the shoulder phase (Table 3.2). Yet, when comparing the difference in R^2 value between Eq. (3.3) and Eq. (3.6), the LLS model fitted better 10 (83.33 %) out of 12 survival curves and for the other two curves, both models have equal R^2 values. However, the difference in R^2 values between the LL and LLS model were very small suggesting that the shoulder phases might not be significant and simply caused by the variation of observed data or model overfitting (Xiong et al., 1999).

Because it is well known that the RMSE and R^2 values are generally not the most adequate indicator when comparing models of dissimilar complexity such as the LL and LLS models evaluated in this study (Kumar et al., 2018), the values of AIC criterion were also analyzed (Table 3.2). The LL model provided very small AICc values compared to the value for the LLS model except for the 394.5 mM ethanol treatment, which had the lowest R^2 (0.894) (Table 3.2). According to this criterion, the LL model better predicted the data for 11 out of 12 (91.67 %) of the curves. Bahceci and Acar (2007) stated that if the R^2 value of the thermal inactivation curve was higher than 0.90, it could be considered linear as found in the present study.

To this point, the LL model provided the better prediction of survival curves in term of AIC whereas the LLS model was better in term of RMSE and R^2. Therefore, to validate the models, it is important that the residuals around the model follow a Gaussian

Table 3.2 RMSE, R^2, and AICc values for the inactivation curves corresponding to *S*. Typhimurium ATCC strain 13311 inactivation in DI water and in different aqueous solutions treated with electron beam irradiation

		LL model (Eq. (3.3))			**LLS model (Eq. (3.6))**		
Treatment[1]	Concentration (mM)	[a]RMSE	[b]R^2	[c]AICc (Eq. (9))	RMSE	R^2	AICc (Eq. (9))
DI Water		0.254	0.987	-0.90	0.113	0.998	17.63
PW		0.164	0.992	-6.13	0.167	0.994	22.36
PBS		0.237	0.980	-1.70	0.086	0.998	14.42
PB	1.0	0.241	0.989	-1.53	0.071	0.999	12.02
	10.0	0.162	0.994	-6.28	0.137	0.997	19.98
	50.0	0.177	0.988	-5.23	0.112	0.996	17.53
Ethanol	78.9	0.162	0.984	-6.24	0.188	0.984	23.75
	394.5	0.166	0.894	-5.99	0.009	1.00	-13.03
	1578.0	0.100	0.929	-12.02	0.066	0.977	11.28
PEG	0.0125	0.191	0.991	-4.29	0.173	0.994	22.82
	0.125	0.153	0.992	-6.93	0.171	0.992	22.67
	1.875	0.089	0.997	-13.43	0.078	0.998	13.27

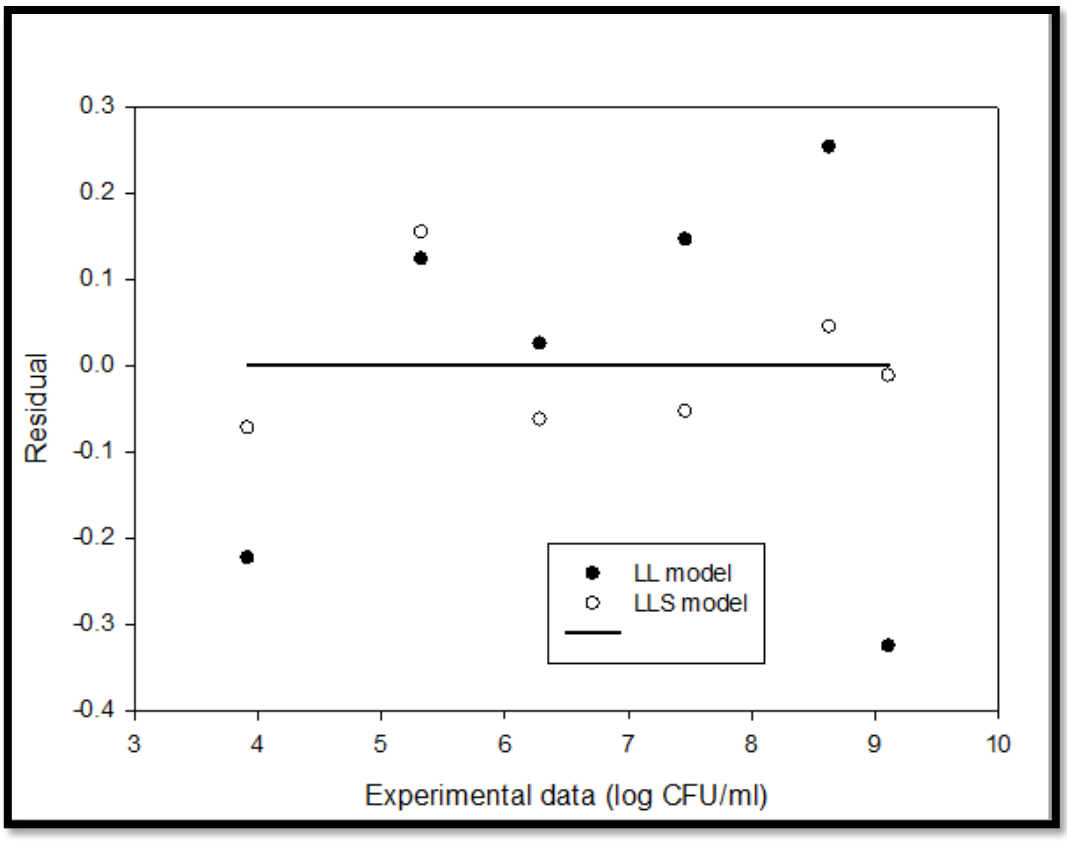

Figure 3.5 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in deionized (DI) water by electron beam irradiation at room temperature

population of model (Kumar et al., 2015). The residuals plots for both models and each aqueous solution are presented in Figures 3.5 and A1-A11 (see Appendix). An example of residuals plot of the both models is depicted on the Fig. 3.5 and the LL. Experimental plots revealed that the LL model yielded R^2 values greater than 0.92 with slope, *a*, ranging from 0.999 to 1.00 while the LLS model yielded R^2 values greater than 0.98 with *a* ranging from 0.999 to 1.00 (Table 3.3). Overall, the LLS model was the model showed slightly higher deviation than the LLS models. In addition, the analysis of predicted vs better than the LL model because it displayed a straighter and less disperse line for all aqueous solutions.

The inactivation rate constant (*k*) values obtained from the LLS model were larger for all aqueous solutions compared to those obtained with the LL model (Table 3.4 and 3.5). The largest (105 %) and lowest (2.61 %) differences on *k* values were observed in the 1578 mM ethanol and 0.125 mM PEG solutions, respectively (Table 3.5). On the other hand, the estimated D_{10} (from the LL model; Eq. (3.5)) and $D_{10\text{- average}}$ (from the LLS model; Eq. (3.7)) displayed different behavior (Tables 3.4 and 3.5). For instance, for the 1578 mM ethanol solution, the estimated $D_{10\text{-average}}$ value decreased by 41.88 % compared to the D_{10} value while the estimated $D_{10\text{-average}}$ value was the same (0.188 kGy^{-1}) as the D_{10} value for the 0.125 mM PEG solution. This result proves that the LL model overestimated the decimal reduction dose because it does not take into account the variability at the cell level due to the effect of complex environments (Bevilacqua et al., 2015).

Table 3.3 R^2 and a value for the predicted vs. observed curves for e-beam primary inactivation models for *S.* Typhimurium ATCC strain 13311 in different aqueous solutions.

		LL model (Eq. (3.3))		**LLS model (Eq. (3.6))**	
Treatment[1]	Concentration (mM)	[a]R^2	[b]a	R^2	a
DI Water		0.987	0.999	0.998	0.999
PW		0.992	1.00	0.992	1.00
PBS		0.98	0.999	0.998	1.00
PB	1.0	0.988	0.999	0.999	1.00
	10.0	0.994	1.00	0.997	1.00
	50.0	0.988	1.00	0.996	1.00
Ethanol	78.9	0.984	1.00	0.984	1.00
	394.5	0.882	1.00	1.00	1.00
	1578.0	0.924	1.00	0.977	1.00
PEG	0.0125	0.991	1.00	0.994	1.00
	0.125	0.992	1.00	0.992	1.00
	1.875	0.998	1.00	0.998	1.00

[1]DI water: deionized water; PW: peptone water; PBS: phosphate buffered saline; PB: phosphate buffer; PEG: polyethylene glycol

[a]R^2: coefficient of determination

[b]a: regression parameter from the equation, $y = a * x$, where, y and x represent predicted and observed values, respectively.

In summary, although both primary inactivation models fitted the survival curves similarly, the LLS model is a better fit based on RMSE, R^2, residuals and observed vs. experiment plots. In addition, the LLS model can fall back into the LL model when the shoulder length, SI, is equal to zero. Therefore, the LLS model is a more general model that should be used to predict the inactivation kinetics of the selected microorganism in irradiated aqueous solutions.

3.4.2 Survival inactivation kinetics parameters based on primary models

Figure 3.6 to 3.10 show the survival curves of *S.* Typhimurium ATCC strain 13311 in DI water or in different aqueous solutions irradiated by e-beam. Based on the conclusion from Section 3.4.1, all further analysis of the radiation inactivation parameters was based on the LLS model results.

The shoulder length, *SI,* and $D_{10\text{-}average}$ values for *S.* Typhimurium ATCC strain 13311 irradiated in DI water by e-beams were 0.098±0.012 and 0.147±0.004, respectively, (Table 3.4). Figure 3.17 shows the e-beam irradiation survival curves for the pathogen in DI water and peptone water including 10 g/1000ml gelatin peptones and 5 g/1000ml NaCI. Over 5-log (99.999 %) reductions of the microorganism in DI water were achieved by using 0.75 kGy dose. However, when the peptone water (PW) was added into the water, the radiation resistance of the pathogen increased ($P < 0.05$) by 19.73 % as the $D_{10\text{-}average}$ increased to 0.176±0.002. Irradiation of the pathogen in this solution decreased ($P > 0.05$) the SI by 51% and significantly ($P < 0.05$) decreased the inactivation rate constant, *k,* (Table 3.4) by 23%. The main explanation for the greater resistance to radiation energy can be explain by the availability of dissolved organic

Table 3.4 Survival kinetics parameters obtained after fitting two different models (log linear (LL) and log-linear plus shoulder (LLS)) for *S.* Typhimurium ATCC strain 13311 in DI water and different aqueous solutions.

		LL model (Eq. (3.3))				**LLS model (Eq. (3.6))**				
Treatment[1]	Concentration (mM)	[a]k (kGy^{-1})	D_{10} (Eq. (3.5)) (kGy)	[b]RMSE	[c]R^2	[d]SI (kGy)	k (kGy^{-1})	$D_{10\text{-average}}$ (Eq. (3.7)) (kGy)	RMSE	R^2
DI Water		$_{w}$16.24 (0.41)*	$_{w}$0.142 (0.004)	0.254	0.987	$_{w}$0.098 (0.012)	$_{w}$18.10 (0.32)	$_{wy}$0.147 (0.004)	0.113	0.998
PW		$_{x}$13.13 (0.18)	$_{x}$0.175 (0.002)	0.164	0.992	$_{w}$0.048 (0.030)	$_{x,y}$13.85 (0.61)	$_{x}$0.176 (0.002)	0.167	0.994
PBS		$_{x}$12.26 (0.36)	$_{y}$0.188 (0.006)	0.237	0.98	$_{w}$0.134 (0.051)	$_{x,y}$14.48 (1.05)	$_{xz}$0.186 (0.005)	0.086	0.998
PB	1.0	$_{w}$16.42 (0.51)	$_{w}$0.140 (0.004)	0.241	0.989	$_{w}$0.095 (0.051)	$_{w}$18.33 (1.59)	$_{w}$0.145 (0.003)	0.071	0.999
	10.0	$_{y}$14.92 (0.19)	$_{z}$0.154 (0.002)	0.162	0.994	$_{w}$0.051 (0.026)	$_{y}$15.77 (0.61)	$_{y}$0.156 (0.002)	0.137	0.997
	50.0	$_{x}$11.69 (0.37)	$_{y}$0.197 (0.006)	0.177	0.988	$_{w}$0.089 (0.041)	$_{x}$13.00 (0.84)	$_{z}$0.195 (0.006)	0.112	0.996

*Standard deviation

$_{w,x,y,z}$ Means within a column, which are not followed by a common subscript letter, are significantly different ($P < 0.05$)

[1]DI water: deionized water; PB: peptone water; PBS: phosphate buffered saline; PB: phosphate buffer

[a]k: rate constant (kGy^{-1}); [b]RMSE: root mean square root; [c]R^2: coefficient of determination; [d]SI: shoulder length

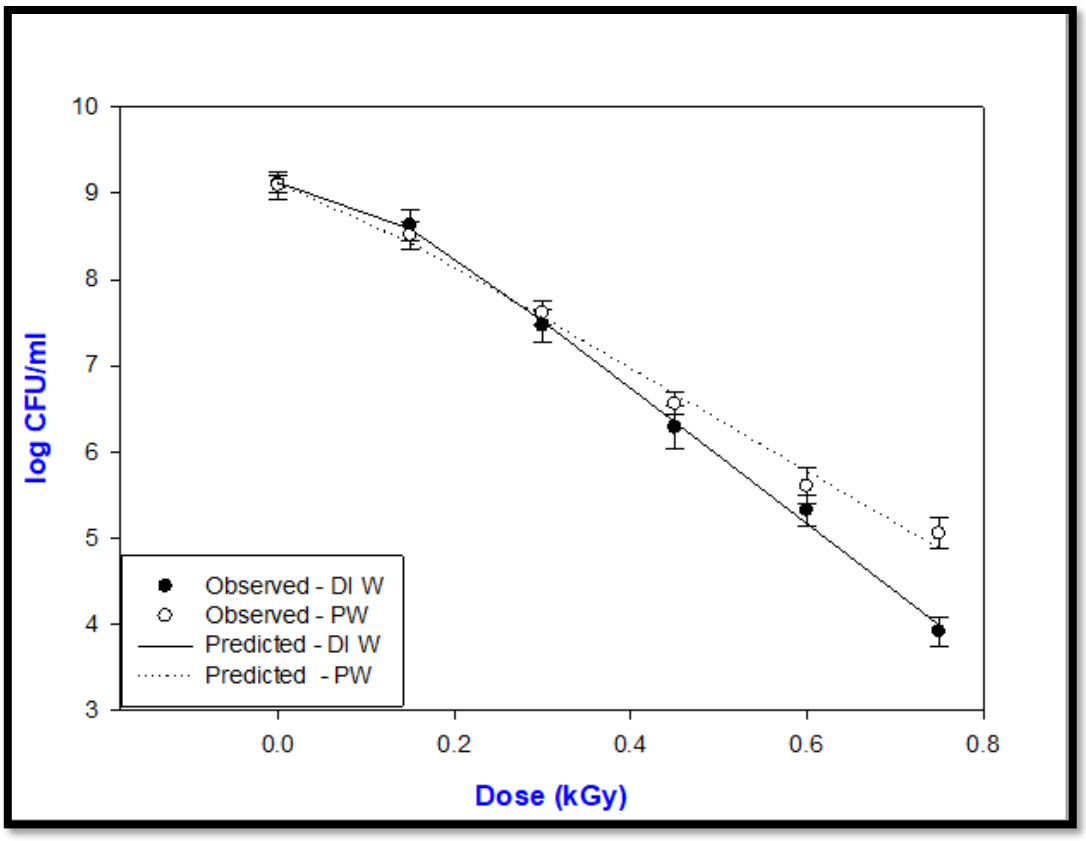

Figure 3.6 Survival curves for *S.* Typhimurium ATCC strain 13311 in DI W (deionized water) and PW (peptone water) using electron beam irradiation at room temperature. Experimental data were fitted by the LLS (log-linear plus shoulder) model.

compounds, since peptone, a complex mixture of peptides with small content of free amino acids (Amezaga and Booth, 1999), can react with hydroxyl radicals formed during radiolysis of water as reported on previous studies (Johansen and Howard-Flanders, 1965; Dion et al., 1994; Van Gerwen et al., 1999). Particularly, amino acids and thiols were reported as radioprotectors (Singh and Singh, 1982). In addition, Ayari et al. (2009) stated that the manufacturing of high molecular weight peptides after irradiation, as presented by PW in the present study, may allow bacteria to maintain its integrity and thus show repairing capabilities in order to survive irradiation stress. Furthermore, it is known that irradiation in a NaCI solution could produce secondary dichloride radicals (Brustad and Wold, 1976; Sanner and Pihl, 1969), which can react with hydroxyl radicals formed in the radiolysis of water during the irradiation process. Thus, these mechanisms may provide a protective effect on *S*. Typhimurium ATCC strain 13311 cells. To best of our knowledge, there are no other studies on the effect of peptone water on the radiation sensitivity of microorganisms for direct comparison with our findings.

Figure 3.18 shows that the addition of phosphate buffered saline (PBS), including 10 mM PB and 150 mM NaCI, into the DI water increased ($P < 0.05$) by 26.53 % the radiation resistance of the pathogen. The calculated *SI* value for S. Typhimurium in PBS was not significantly ($P > 0.05$) different from that for DI water whereas the calculated *k* value for this organism was significantly ($P < 0.05$) different those of DI water (Table 4). The $D_{10\text{-average}}$ value for *S*. Typhimurium ATCC 13311 in DI water with PBS was about 0.186 ± 0.005 kGy (Table 4). This result agrees with the D_{10} value obtained by Underdal

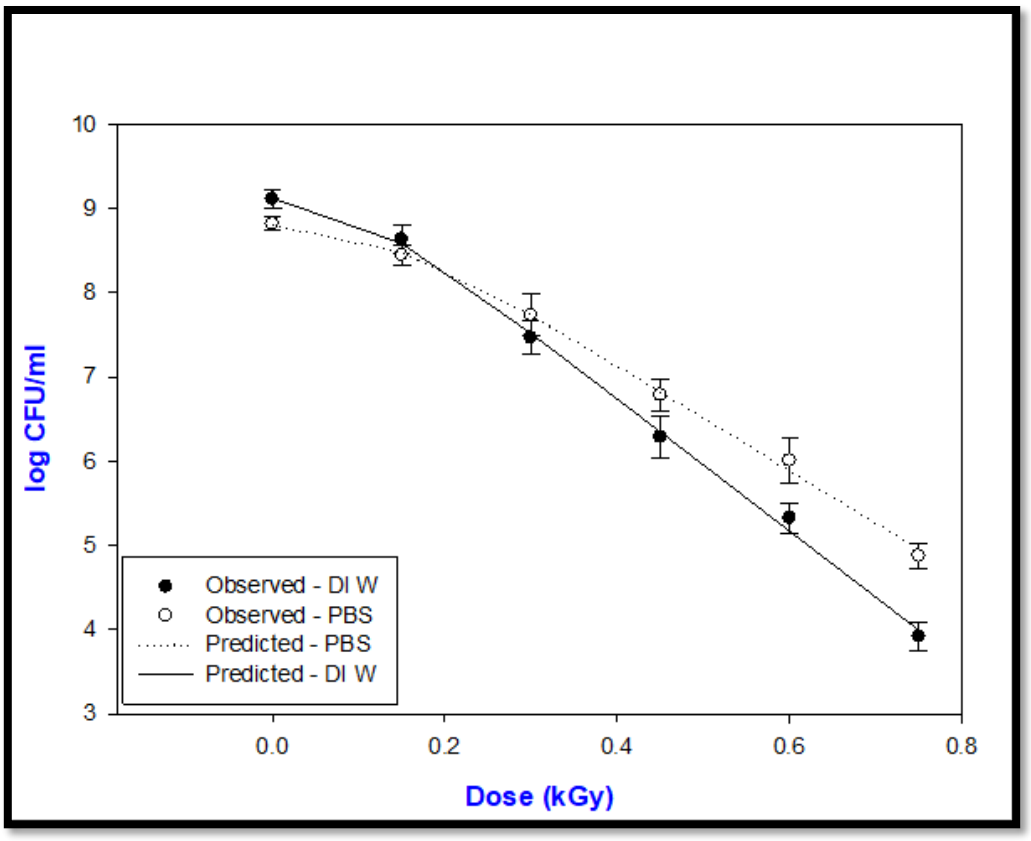

Figure 3.7 Survival curves for *S.* Typhimurium ATCC strain 13311 in DI W (deionized water) and PBS (phosphate buffered saline) using electron beam irradiation at room temperature. Experimental data were fitted by the LLS (log-linear plus shoulder) model

and Rossebo (1972), which ranged from 0.170 kGy for *S.* Senftenberg strain 54 to 0.232 kGy for *S.* Senftenberg strain 775W when irradiated in buffer by gamma rays. On the other hand, Dion et al. (1994) reported D_{10} values for *S.* Typhimurium strain 14028 in 150 mM saline solution as 0.14 kGy. The difference with the D_{10} values from this study are probably due to the presence of phosphate buffer (PB). Similarly, a study compared the radiation sensitivity of *E. coli* in NaCI and NaCI plus PB (67 mM) solutions during gamma irradiation and found that radiaosensitivity of the bacterial population in the NaCI solution was higher ($P < 0.05$) than that in NaCI plus PB (Namiki et al., 1961). Furthermore, Borrely et al. (1998) pointed out that the D_{10} value for *S.* Typhimurium irradiated in buffer solution was 0.30 kGy. This value is much higher than those of in the present study possibly due to the radiation sensitivity differences between *S.* Typhimurium strains as reported by Underdal and Rossebo (1972) and the amount of compounds in buffer solution, which were not reported by Borrely et al. (1998).

The inactivation characteristics of *S.* Typhimurium ATCC strain 13311 were examined at three different PB concentrations (1, 10, and 50 mM) and compared with those for DI water (Figure 3.19). The calculated SI values for *S.* Typhimurium ATCC strain 13311 were not significantly ($P > 0.05$) affected by the PB concentration level or for irradiation in DI water alone (Table 3.4). Furthermore, the calculated *k* value in 1 mM PB was not different ($P > 0.05$) from that for DI water, but the higher concentrations of PB decreased the reaction rates significantly ($P < 0.05$) (Table 4). Overall, the calculated $D_{10\text{-average}}$ values for *S.* Typhimurium ATCC strain 13311 in DI water increased ($P < 0.05$) by with increasing PB concentration. Hence, irradiation in

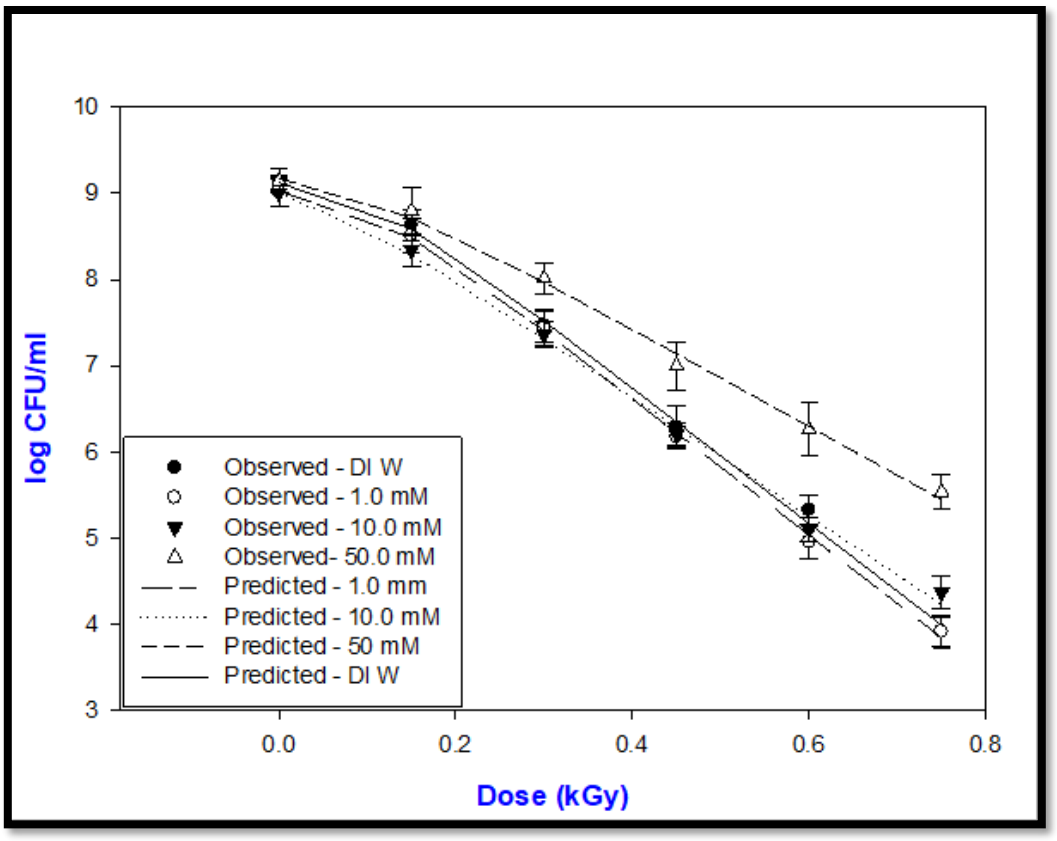

Figure 3.8 Survival curves for *S.* Typhimurium ATCC strain 13311 in DI W (deionized water) and various concentration of PB (phosphate buffer) using electron beam irradiation at room temperature. Experimental data were fitted by the LLS (log-linear plus shoulder)

aqueous solutions with 10 and 50 mM PB decreases ($P < 0.05$) the radiation sensitivity of the selected pathogen.

There are two possible mechanisms to explain these findings. First, the probability that OH-induced hydrogen abstraction from phosphate occurred (Brustad and Wold, 1976) and the phosphate buffer reduced the yield of the hydroxyl radical formed from the radiolysis of water during e-beam irradiation. Second, the increased concentration of potassium (K^+) inhibited the radiation sensitivity of *S*. Typhimurium ATCC strain 13311 during e-beam irradiation treatment due to potassium cation transporters and channels, which allow molecular adaption to various environmental conditions (Epstein, 2003; Rincon and Pulgarin, 2004).

The radiation sensitivity of *S*. Typhimurium ATCC strain 13311 in 10 mM PB also decreased ($P < 0.05$) by 19.23 % when 10 mM PB was supplemented with 150 mM NaCI; eg, the PBS solution used in this study (Table 3.4). This result can be due to the reaction of the chloride anion (CI^-) with hydroxyl radicals (Ward and Myers, 1965). On the other hand, Czapski et al. (1992) determined that the production of hypochlorite in N_2O-saturated solutions of PBS (42 mM PB and 250 mM NaCI) was linear with radiation dose and the viability of *E. coli* cells in this solution declined sharply with radiation in a dose-dependent response like the concentration response of this organism cells to commercial NaOCI. This difference with our result is probably due to the initial cell concentration used as Matsuyama et al. (1960) concluded that with a considerably high cell concentration (about 9 log CFU/ml as used in the present study), NaCI decreased the radiation lethal effect in contrast with the case of low concentrations.

Shamsuzzaman et al. (1989) evaluated the radiation sensitivity of nalidixic acid resistant strain *S.* Typhimurium Nal^R ATCC strain 13311 in a 67 mM PB solution and determine a D_{10} value of 0.198±0.013 kGy, which is close to the D_{10} value (average 0.195±0.006 kGy) for 50 mM PB determined in the present study. Accordingly, Thayer et al. (1990) reported a D_{10} value of 0.199±0.013 kGy for *S.* Typhimurium ATCC strain 14028 irradiated in 50 mM PB by cesium-137 gamma radiation source.

3.4.3 Significance of the hydroxyl radical on S. Typhimurium inactivation using electron beam irradiation

Figure 3.20 shows the survival curves of *S.* Typhimurium in ethanol solutions as a function of the absorbed dose. Although irradiation in the 78.9 mM ethanol concentration decreased ($P < 0.05$) the shoulder length, *SI*, by 68.42 %, the higher ethanol concentrations (394.5 and 1578 mM) increased ($P > 0.05$) the *SI* values by 336.84% and 325.26 %, respectively (Table 3.5). Moreover, the inactivation rate constant, *k*, of *S.* Typhimurium in DI water including 1 mM PB decreased ($P < 0.05$) by 46.15 %, 57.56 %, and 70.43 % when the concentration of ethanol in the solution was modified as 78.9, 394.5, and 1578 mM (Table 3.5). Overall, the radiation sensitivity of *S.* Typhimurium in 78.9 mM, 394.5 mM, and 1578 mM decreased ($P < 0.05$) by 65.51 %, 162.07 %, and 250.34 % compared with those of DI water including 1 mM PB (Table 3.5). These results confirmed that the presence of hydroxy radical scavenger in aqueous solution at room temperature (~22 °C) reduced radiation-induced cell lethality of *Salmonella* Typhimurium as reported at 0°C (Kim and Thayer, 1995).

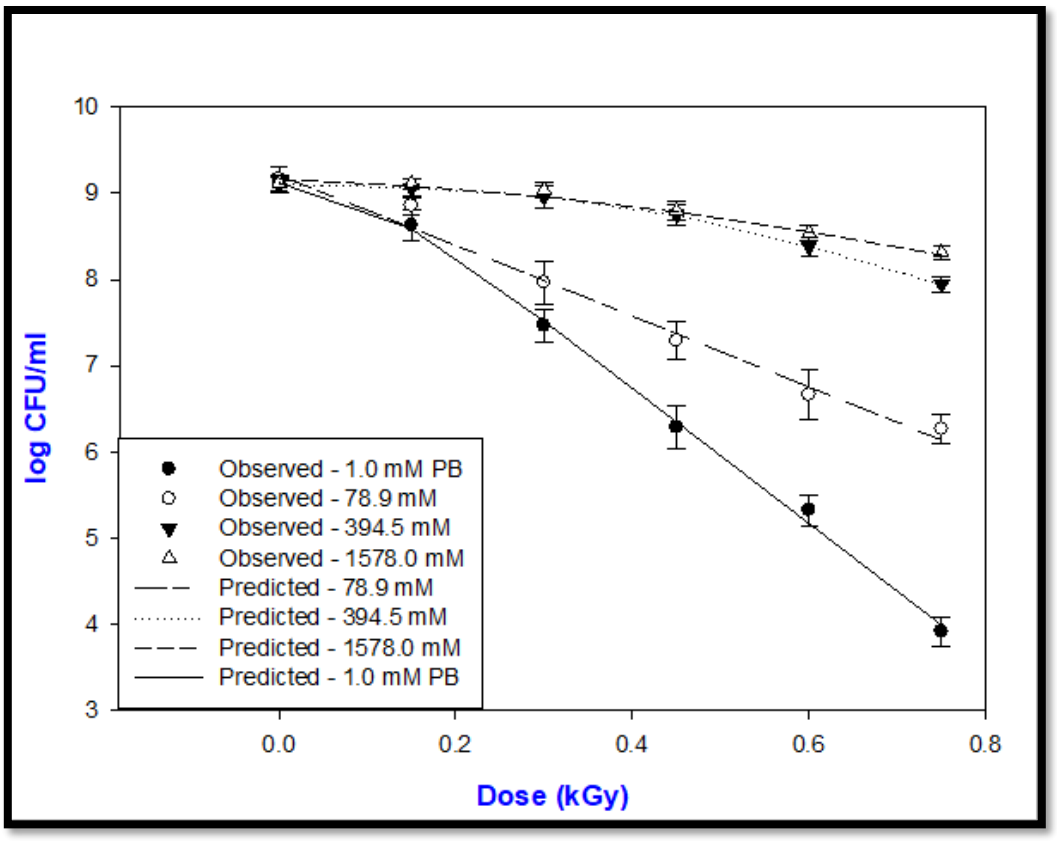

Figure 3.9 Survival curves for *S.* Typhimurium ATCC strain 13311 in 1.0 mM PB (phosphate buffer) and various concentration of EtOH (ethanol) using electron beam irradiation at room temperature. Experimental data were fitted by the LLS (log-linear plus shoulder)

Table 3.5 Survival kinetics parameters obtained after fitting two different models (log linear (LL) and log-linear plus shoulder (LLS)) for *S.* Typhimurium ATCC strain 13311 in different aqueous solutions.

		LL model (Eq. (3.3))				**LLS model (Eq. (3.6))**				
Treatment [1]	Concentration (mM)	[a]k	D_{10} (Eq. (5))	[b]RMSE	[c]R^2	[d]SI	k	$D_{10\text{-average}}$ (Eq. (7))	RMSE	R^2
PB	1.0	$_{u}$16.42 (0.51)*	$_{u}$0.140 (0.004)	0.241	0.989	$_{u}$0.095 (0.051)	$_{u}$18.33 (1.59)	$_{u}$0.145 (0.003)	0.071	0.999
Ethanol	78.9	$_{v}$9.54 (0.67)	$_{u}$0.242 (0.018)	0.162	0.984	$_{u}$0.03 (0.032)	$_{v}$9.87 (0.36)	$_{v}$0.240 (0.014)	0.188	0.984
	394.5	$_{w}$3.54 (0.40)	$_{v}$0.656 (0.079)	0.166	0.894	$_{v}$0.415 (0.039)	$_{w}$7.78 (0.66)	$_{w}$0.380 (0.024)	0.009	1.00
	1578.0	$_{w}$2.64 (0.020)	$_{w}$0.874 (0.007)	0.100	0.929	$_{v}$0.404 (0.031)	$_{x}$5.42 (0.54)	$_{x}$0.508 (0.003)	0.066	0.977
PEG	0.0125	$_{x}$14.60 (0.42)	$_{x}$0.158 (0.004)	0.191	0.991	$_{u}$0.055 (0.031)	$_{y}$15.52 (0.83)	$_{u}$0.160 (0.004)	0.173	0.994
	0.125	$_{y}$12.26 (0.39)	$_{y}$0.188 (0.006)	0.153	0.992	$_{u}$0.024 (0.032)	$_{z}$12.58 (0.28)	$_{y}$0.188 (0.006)	0.171	0.992
	1.875	$_{y}$11.00 (0.77)	$_{y}$0.210 (0.014)	0.089	0.997	$_{u}$0.061 (0.051)	$_{z}$11.84 (1.26)	$_{v}$0.208 (0.013)	0.078	0.998

*Standard deviation

$_{u,v,w,x,y}$: Means within a column, which are not followed by a common subscript letter, are significantly different ($P < 0.05$)

[1]PB: phosphate buffer; PEG: polyethylene glycol

[a]*k*: rate constant (kGy^{-1}); [b]RMSE: root mean square root; [c]R^2: coefficient of determination; [d]SI: shoulder length

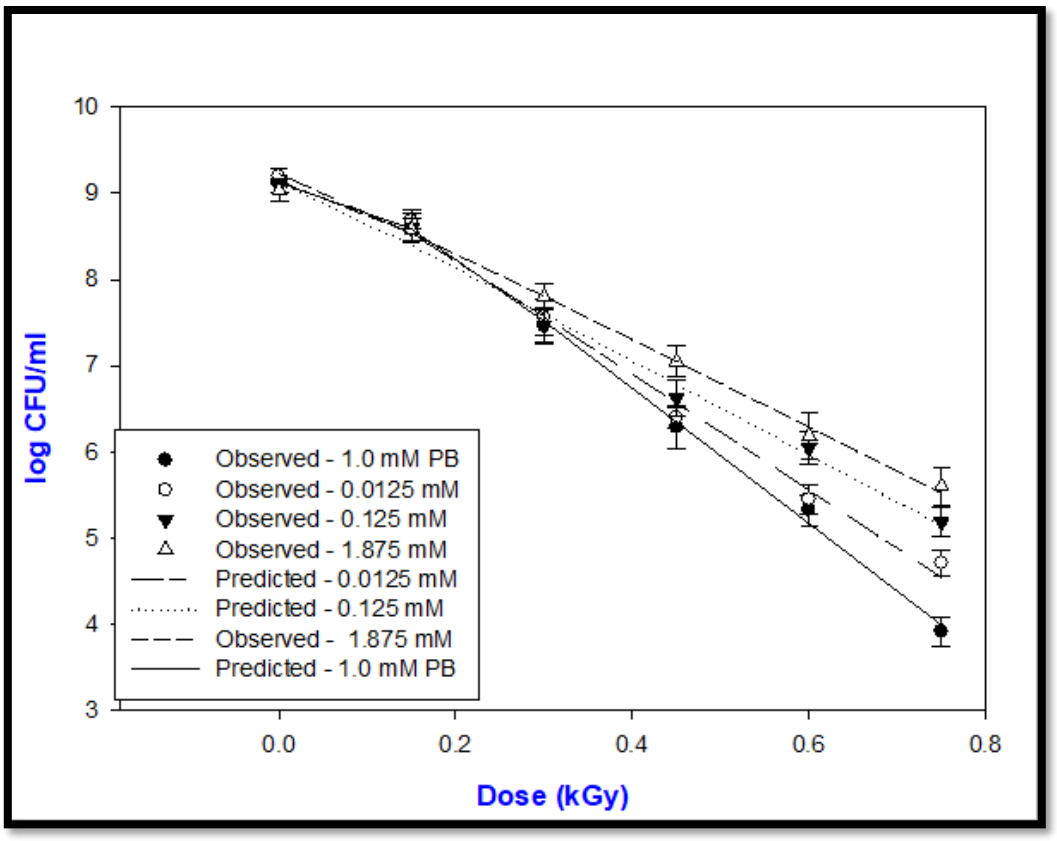

Figure 3.10 Survival curves for *S.* Typhimurium ATCC strain 13311 in 1.0 mM phosphate buffer (PB) and various concentration of PEG (polyethylene glycol) using electron beam irradiation at room temperature. Experimental data were fitted by the LLS (log-linear plus shoulder)

Figure 3.21 shows the effect of polyethylene glycol (PEG), a membrane-impermeable hydroxyl radical scavenger, on the survival curves of *S.* Typhimurium. Concentration levels of PEG did not ($P > 0.05$) affect the SI values (Table 5). On the other hand, the *k* values decreased ($P < 0.05$) by 15. 33 %, 31.37 %, and 35.41 % for 0.0125 mM, 0.125 mM, and 1.875 mM, respectively, compared with the *k* values for DI water and 1 mM PB solution (Table 3.5). Therefore, the radiation resistance of *S.* Typhimurium in DI water with 1 mM PB increased ($P < 0.05$) up to 43.45 % when the concentration of PEG increased in the range used in this study (Table 3.5).

These results suggest that the availability of hydroxyl radical scavengers is effective in protecting the microbial cells during irradiation and the greatest inactivation of this organism was associated with indirect rather than direct radiation damage as reported by others (Kim and Thayer, 1995; Siddiqi and Bothe, 1987; van Sonntag, 2006). Furthermore, Roots and Okada (1972) stated that although the level of protection supplied by these scavengers increased as their concentrations increased, it always only up to a certain maximum as seen in Figure 3.11 and 3.12 in the present study. The higher concentrations of ethanol (394.5 mM and 1578 mM) yielded a higher ($P < 0.05$) shoulder indicating that the hydroxyl radical scavenger enhanced the resistance of the pathogen to ionizing radiation both by removing hydroxyl radicals and protecting against oxygen-dependent damage (Ewing and Kubala, 1987).

The type of scavenger did not affect ($P > 0.05$) the SI values of *S.* Typhimurium at all concentration levels studied but the *k* value for ethanol solutions was lower ($P < 0.05$) than those of PEG (Table 3.5), confirming that most of the inactivation of this

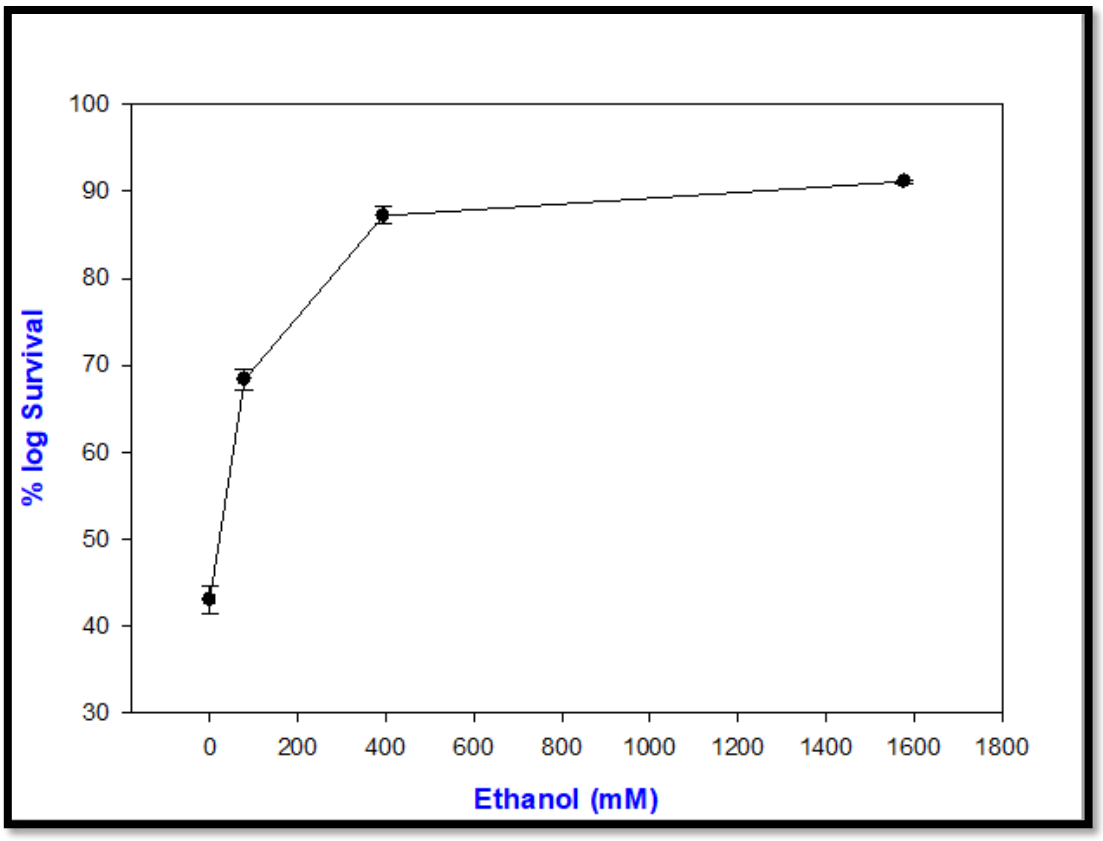

Figure 3.11 Survival of *S*. Typhimurium ATCC strain 13311 in air saturated 1.0 mM phosphate buffer (PB) including various concentration of ethanol.

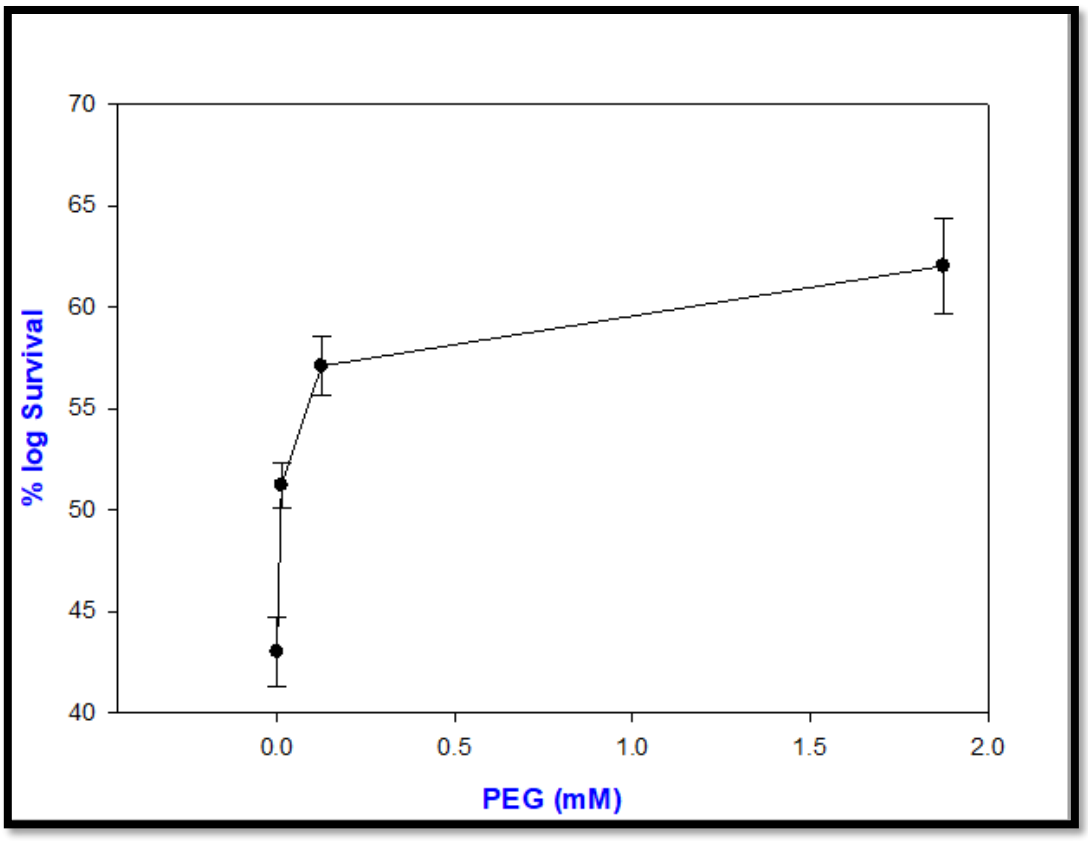

Figure 3.12 Survival of *S*. Typhimurium ATCC strain 13311 in air saturated 1.0 mM PB (phosphate buffer) including various concentration of polyethylene glycol (PEG).

pathogen occurred due to intracellular hydroxyl radicals. However, Kim and Thayer (1995) suggested that the most lethality of *S*. Typhimurium in PB was linked to extracellular hydroxyl radicals. This difference with our result is due to the use of different concentrations of membrane-permeable hydroxyl scavenger even though the reaction rate constant for the formate ion with hydroxyl radical is higher than that for ethanol with hydroxyl radical (Buxton et al., 1988).

Ewing and Kubala (1987) also reported that the high concentrations of methanol, ethanol, glycerol, and DMSO (dimethyl sulfoxide) could be able to protect *E. coli* B/r cells in 0.067 M PB irradiated by X-rays against oxygen-dependent damage. Likewise, Siddiqi and Bothe (1987) found that the yield of double-strand breaks (DSBs), which are lethal if unrepaired, in calf thymus DNA in N_2O containing oxygenated aqueous solution reduced while the concentration of hydroxyl radical scavengers in the solution increased. Accordingly, Singh and Apte (2018) showed that both radiation-induced single-strand breaks (SSBs) and DSBs in plasmid pBlueescript DNA suspended in 1.0 mM EDTA solution (pH=8.0) and exposed to gamma radiation were reduced when ethanol concentration was raised.

In summary, the presence of hydroxyl radical scavengers increases the resistance of the studied pathogen to ionizing radiation at the dose levels of this study and ethanol is more effective than PEG and the most lethality is associated with intracellular hydroxyl radicals and indirect rather than direct radiation damage.

3.4.4 Secondary models for inactivation of S. Typhimurium in various aqueous solutions

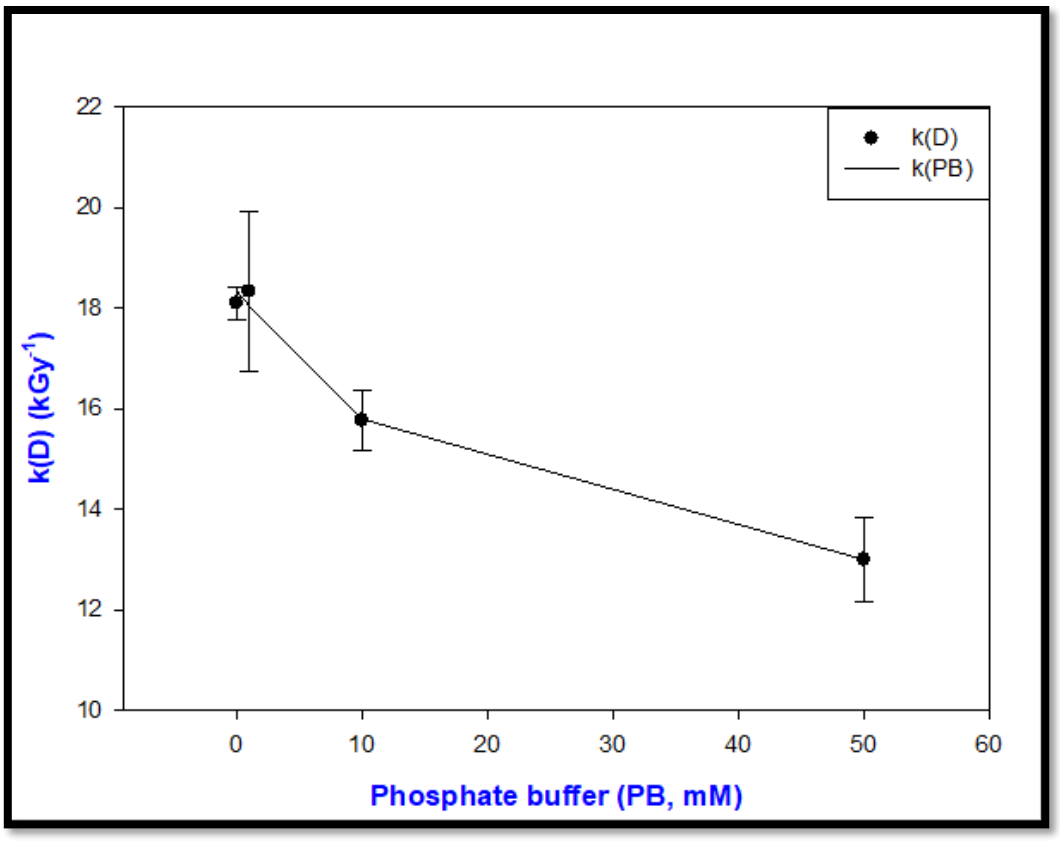

Figure 3.13 Calculated k(D) values from the log-linear plus shoulder (LLS) model (Eq. (3.6)) for *S.* Typhimurium ATCC strain 13311 in an aqueous solution and predicted inactivation rate constant as a function of the concentration of phosphate buffer (PB).

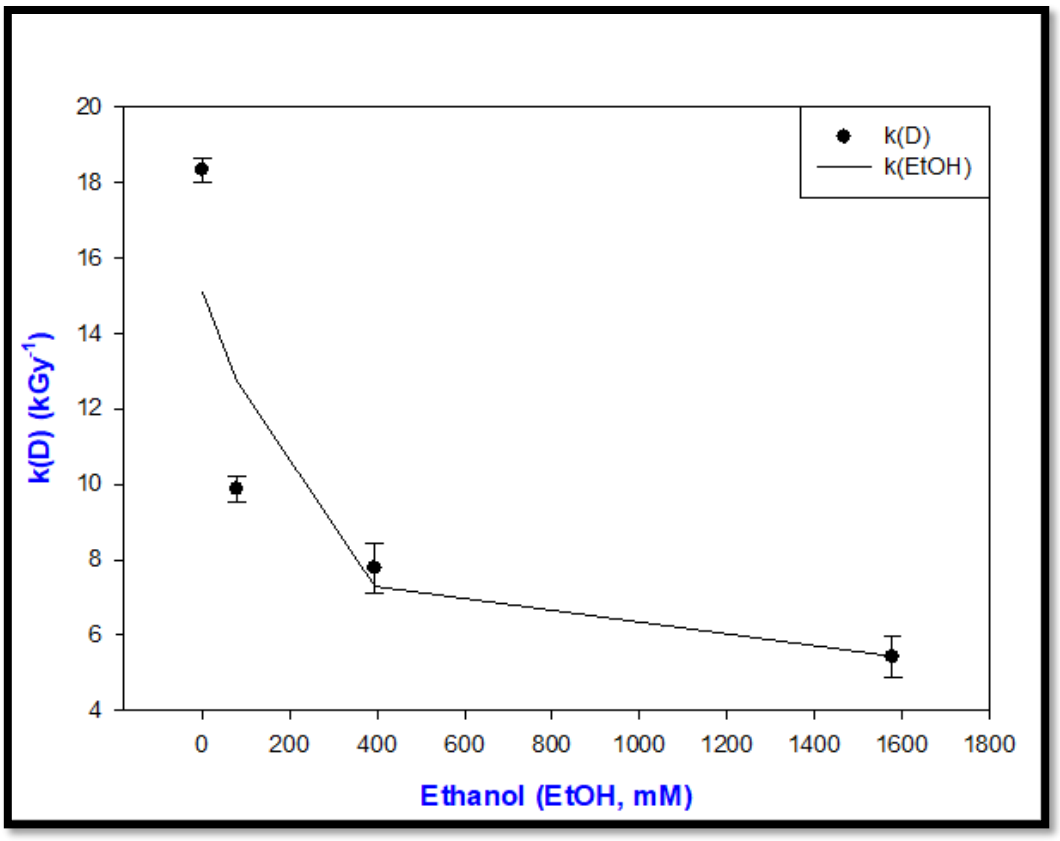

Figure 3.14 Calculated k(D) values from the log-linear plus shoulder (LLS) model (Eq. (3.6)) for *S.* Typhimurium ATCC strain 13311 in an aqueous solution and predicted inactivation rate constant as a function of the concentration of ethanol (EtOH).

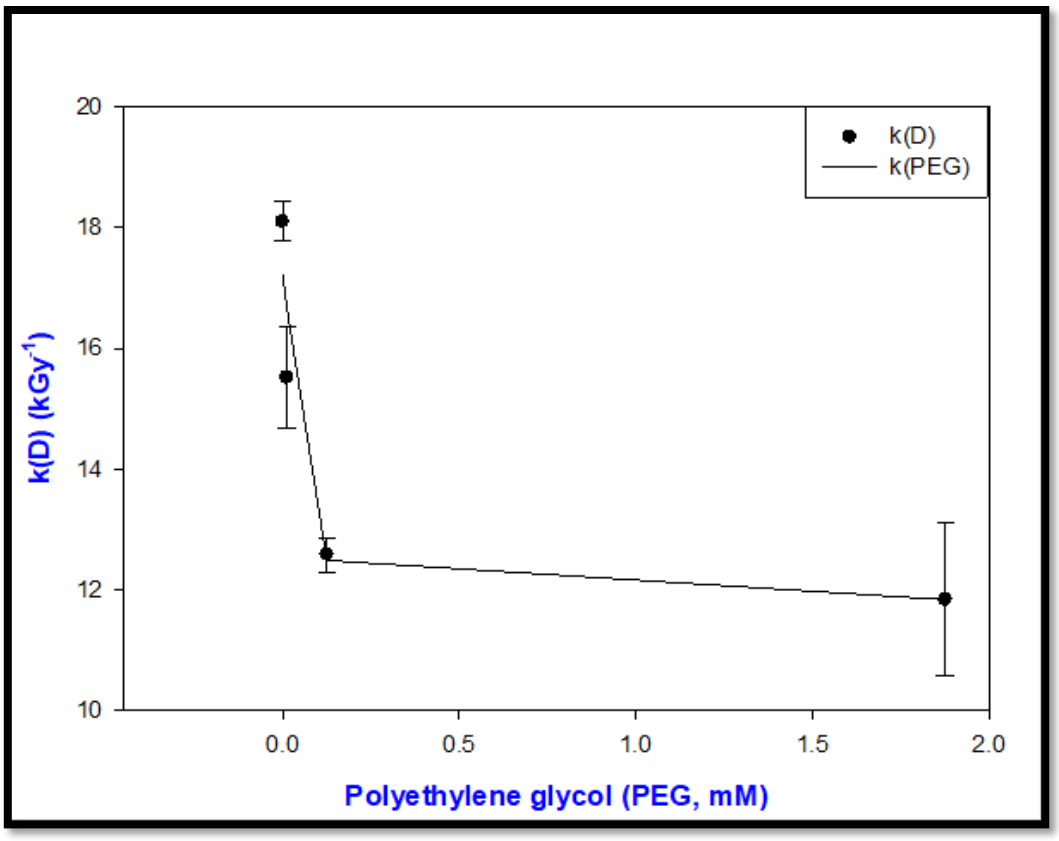

Figure 3.15 Calculated k(D) values from the log-linear plus shoulder (LLS) model (Eq. (3.6)) for *S.* Typhimurium ATCC strain 13311 in an aqueous solution and predicted inactivation rate constant as a function of the concentration of polyethylene glycol (PEG)

Table 3.6 Coefficients of Eq. (3.8) used to estimate the values of inactivation rate constant (k(D)) obtained from the fit of log-linear plus shoulder model (LLS; Eq. (3.6)) as a function of the concentration of chemical agent in 1 mM phosphate buffer (PB) for *S*. Typhimurium ATCC strain 13311 in aqueous solutions

Parameter /Treatment[1]	**[a]A_1**	**95% CI**	**[b]A_2**	**95% CI**	**[c]A_3**	**95% CI**	**[d]R^2**	**[e]RMSE**	**A_f (Eq. (3.10))**	**B_f (Eq. (3.11)**
PB	-2.91	(-3.11, -2.71)	0.017	(-0.026, 0.0.60)	-2.02E-4	(-1.0E-3, 6.1E-4)	0.994	0.021	1.00	1.00
Ethanol	-2.72	(-6.05, 0.62)	0.002	(-0.016, 0.021)	-1.01E-6	(-1.2E-5, 1.0E-5)	0.865	0.326	1.14	1.00
PEG	-2.85	(-3.74, -1.95)	2.74	(-10.46, 15.93)	1.35	(-8.24, 5.54)	0.957	0.064	1.04	1.00

[1]PB: phosphate buffer; PEG: polyethylene glycol

[a,b,c]A_1, A_2, and A_3, regression parameters of Eq. (9); [d]R^2: coefficient of determination; [b]RMSE: root mean square error

Figures 3.13 to 3.15 show the effect of the concentration of PB, ethanol, and PEG on the inactivation rate constant (*k*) estimated from the log-linear plus shoulder (LLS) model (Eq. (3.6)). Table 3.6 shows the good fit provided by Eq. (3.8) with 95 % CI. The R^2 and RMSE ranged from 0.865 to 0.997 and 0.015 to 0.326, respectively. In addition, A_f values varied from 1.00 to 1.14 (Table 3.6), confirming the suitability of the model (Hwang and Tamplin, 2007). Furthermore, Zhou et al. (2014b) justified that when the B_f factor used as criterion for validation was between 0.75 and 1.25, it can prove the validation of the microbial models. B_f values of 1.00 were obtained in this study. Hence, the A_f and B_f obtained in the present work were in a safe and acceptable range. Thus, these results confirm the good agreement between the observed and predicted values for the inactivation rate constant, *k*, using the dynamic models. The relationship between inactivation rate constant and concentration of PB (Eq. (3.12)), ethanol (EtOH, Eq. (3.13)), and PEG (Eq. (3.14)) for *S*. Typhimurium, respectively, were:

$$k(PB) = \frac{1}{e^{(-2.91+0.017*C-2.02*10^{-4}*C^2)}} \quad [3.12]$$

$$k(EtOH) = \frac{1}{e^{(-2.72+0.002*C-1.01*10^{-6}*C^2)}} \quad [3.13]$$

$$k(PEG) = \frac{1}{e^{(-2.85+2.74*C-1.35*C^2)}} \quad [3.14]$$

3.5 Conclusion

In the present study, the radiation sensitivity of *S*. Typhimurium ATCC strain 13311 in air-saturated DI water and in different aqueous solutions was investigated. The results revealed that although both the log-linear (LL) and log-linear plus shoulder (LLS) model produced similar survival curves, the LLS model is more general and has the capability

to simulate linear as well as curves with the presence of the shoulder (lag phase). Residual analysis showed that the LLS model was better and more robust than the LL model. Therefore, the LLS model can help estimate the appropriate dose required for the inactivation of the selected pathogen when irradiated in aqueous solutions by using e-beam at the dose level tested in this study.

The addition of peptone water (PW), and phosphate buffered saline (PBS) significantly ($P < 0.05$) affected the radiation sensitivity of *S.* Typhimurium in air saturated deionized (DI) water during e-beam irradiation. Furthermore, the presence of 0.15 mM NaCI and increased concentration of phosphate buffer raised the radioresistance of this pathogen in DI water. On the other hand, the shoulder length, *SI*, values of *S.* Typhimurium irradiated in air saturated DI water were not affected ($P > 0.05$) by the presence of these chemical agents. Likewise, the inactivation rate constant, *k*, for S. Typhimurium in 1.0 mM PB solution was not ($P > 0.05$) different than that in DI water. As a result, this study demonstrated that culture media used for inoculum preparation in microbial studies could significantly affect the radiation sensitivity of pathogens in foods as reported by Moreira et al. (2012). Hence, to minimize the protection effect of these media from irradiation treatment, 1.0 mM PB can be used in microbial studies.

The presence of hydroxyl radical scavengers, ethanol (78.9, 394.5 and 1578 mM) and PEG (0.125 and 1.875 mM) decreased ($P < 0.05$) the radiation sensitivity of *S.* Typhimurium in air saturated DI water with 1.0 mM PB. The addition of membrane-permeable ethanol provided better protection than non-permeable PEG. This result

suggests that the most inactivation of this pathogen occurred due to intracellular hydroxyl radicals. Moreover, the SI values of *S.* Typhimurium in 1.0 mM PB buffer solution significantly ($P < 0.05$) increased by 336.84 % and 325.26 % with the addition of 394.5 mM and 1578.0 mM ethanol.

In summary, the effectiveness of electron beam irradiation against *S.* Typhimurium in aqueous solution was affected by the preinoculation culture for inoculum and the presence of hydroxyl radical scavengers in the solution. Results from this study revealed that the presence of compounds reacting with hydroxyl radical in aqueous solution should consider when testing of the efficacy of ionizing radiation against pathogens in aqueous solution including fresh produce wash water. Further research is needed to increase the yield of hydroxyl radicals in aqueous solution during ionizing radiation in order to enhance the effectiveness of this treatment against inactivation of pathogens on fresh produce.

CHAPTER IV

THE EFFECT OF WATER QUALITY PARAMETERS AND ADDITION OF HYDROGEN PEROXIDE ON THE EFFECTIVENESS OF ELECTRON BEAM IRRADIATION FOR INACTIVAION *SALMONELLA* TYPHIMURIUM ATCC 13311 IN AQUEOUS SOLUTIONS

4.1 Overview

The water quality parameters pH, nitrate, dissolved organic carbon (DOC), and alkalinity) can affect the yield of hydroxyl radical (˙OH) produced from radiolysis of water via ˙OH-scavenging reactions. This study evaluated whether these parameters had an impact on the efficiency of electron beam (e-beam) treatment and a combination of e-beam with hydrogen peroxide (H_2O_2) to inactivate *Salmonella* Typhimurium ATCC strain 13311 in aqueous solution. The changes in the pH value (5.5-8.5) and various concentration (50, 200, and 500 mg/l) of calcium carbonate ($CaCO_3$) did not affect ($P > 0.05$) the inactivation rate constant of the pathogen in aqueous solutions. Addition of 100 mg/l and 1000 mg/l of nitrate decreased ($P < 0.05$) the predicted $D_{10\text{-}average}$ values by 6.21 % and 11.72 %, respectively, indicating that the dissolved oxygen in buffer solution is not sufficient to react with all hydrated electrons formed from radiolysis of water and, the addition of hydrated electron scavengers enhance the effectiveness of e-beam treatment. In addition, the predicted $D_{10\text{-}average}$ values for the pathogen in aqueous solution decreased ($P < 0.05$) by 8.28 % and 11.03 % when 10 mg/l and 100 mg/l of fulvic acid (FA) were added into the buffer solution.

In the present study, e-beam treatment with a dose of 0.75 kGy decreased the population of S. Typhimurium in buffer solution by 5.10±0.019 log CFU/ml whereas exposure of the pathogen in aqueous solution with different concentrations of H_2O_2 to a dose of 0.60 kGy decreased the microbial populations by 6.44±0.031 log CFU/ml, regardless of H_2O_2 concentration. These results confirm that the combination of e-beam treatment with H_2O_2 in aqueous solution increases the radiation sensitivity of the pathogen and provides an alternative treatment strategy to treat contamination and prevent cross contamination of fresh produce washing water. In addition to the great value of preventing foodborne illness outbreaks, the application of this hurdle technology should decrease processing cost due to the reduction in required dose and amount of H_2O_2 concentration needed for 5-log microbial reductions. Furthermore, this technology can reduce and/ or replace the use of chlorine to minimize its environmental and public health impacts.

4.2 Introduction

In recent years, the concerns regarding safety of fresh fruit and vegetables have increased because of the raising frequency of documented outbreaks linked to these products (Goodburn and Wallace, 2013; Gould et al., 2017; Wadamori et al., 2017; Bennett et al., 2018). It is difficult to ensure the safety of fresh produce for consumers because they only undergo minimal processing (Gil et al., 2009; Murray et al., 2017). Consequently, the fresh produce industry relies on the use of a large quantity of clean water to minimize the microbial risk as well as to remove dirt and other debris (Banach et al., 2015; Ignat et al., 2015). Due to the large amount of water required in the water

steps, water reuse is recommended by the United States Department of Agriculture (USDA) and the European Union (EU) (Selma et al., 2008). Nevertheless, re-using processing water can serve as a vehicle for dispersion of microorganisms, including pathogens such as *Salmonella, Escherichia coli,* and *Listeria monocytogenes* (Banach et al., 2015; Huang et al., 2018b). Hence, sanitizing agents are used to keep up with the quality of the water and avoid cross contamination of the produce despite their limited direct microbial benefit on the produce (Bermudez-Aguirre and Barbosa-Canovas, 2013; Banach et al., 2015).

Several sanitizing agents have been studied for disinfecting water and surface decontamination purposes (Goodburn and Wallace, 2013; Ramos et al., 2013; Meireles et al., 2016; Khan et al., 2017a). Chlorine is generally used as a water disinfectant in the fresh produce industry because of its ease of use and relative low cost (Banach et al., 2015; Meireles et al., 2016). However, even at high levels (100-200 ppm), it has been shown to have only restrictive effects in decreasing the levels of pathogens on contaminated fresh produce due to a number of factors, such as contact time, temperature, pH, and water properties (Van Haute et al., 2013; Lopez-Galvez et al., 218). The use of high concentration of chlorine with high total organic carbon (TOC) content can also generate unacceptably high levels of trihalomethanes (THMs) and other carcinogenic disinfection byproducts (Gomez-Lopez et al., 2017). In addition, Kettlitz et al. (2016) reported that the concentration of chlorate in 24.5 percent of plant-derived foods-mainly fruits and vegetables were over 0.01 mg/kg, the allowed maximum residue limit in EU, in the German market. Accordingly, Gil et al. (2016) found that the

increased chlorine dose in the processing wash water for fresh produce (due to high concentration of organic matter) increased the accumulation of chlorite. Furthermore, chlorine is not effective against pathogens already internalized in the stomata, vasculature, cut edges, intercellular tissue, and so on (Meireles et al. 2016). Therefore, new alternatives are needed to prevent transfer of microbial pathogens via wash water and to eliminate these pathogens in fresh produce without the current problems linked to the use of chlorine (Murray et al., 2017).

The ionizing radiation process produced from machinery, such as electron beams (e-beam) and X-rays, and natural source including cobalt-60 and cesium-137 has been studied for wastewater and drinking water disinfection treatment (Kurucz et al., 1995; Borrely et al., 1998; Pikaev, 2000; Sommer et al., 2001; Melo et al., 2008; Wang and Chu, 2016)

Food irradiation is an effective process to achieve pathogen inactivation (Gomes et al., 2008; Gomes et al., 2011; Karagoz et al., 2014; Joshi et al., 2018), prevent significant losses (Villa-Rodriguez et al., 2015; Pinela and Ferreira, 2017) and extend shelf-life of fresh produce (Fernandes et al., 2012; Yurttas et al., 2014; Tong et al., 2018). Irradiation is a fast and clean process without chemical addition for oxidation and has an advantage in reducing the required energy cost (Kim et al., 2017). The effectiveness of electron beams has been studied worldwide, including facilities in the U.S., Canada, and Japan for the destruction of organic contaminants and inactivation of pathogenic microorganisms in wastewater and municipal sewage sludge (Hossain et al.,

2018). Taghipour (2004) determined that an electron beam doses of 0.55 kGy was needed to accomplish 4-log inactivation of *E. coli* in wastewater.

It is known that the inactivation of microorganisms was dominated by reactive species generated by water radiolysis mainly due to hydroxyl radical (˙OH) as found in Chapter III (Section 3.4.3). The average yield (G-value) for each reactive species is indicated in brackets in Equation (4.1) below, which display the number of molecules formed per 100 eV energy of absorbed at a pH range of 6.0-8.5 (Wang et al., 2019).

$$H_2O \xrightarrow{radiolysis} [0.28] \cdot OH + [0.27]e_{aq}^{-} + [0.06] \cdot H + [0.07]H_2O_2 + [0.27]H_3O + [0.05]H_2 \qquad [4.1]$$

The pH value may have an important impact on the radiation-chemical yield (Eq. (4.1)) of reactive species formed from radiolysis of water (Wang and Wang 2018). Therefore, the scavengers present in the water system should be take into consideration when e-beam process is designed for water disinfection in fresh produce industry.

Water quality parameters, such as alkalinity, nitrate (NO_3^-), organic matters, and pH, may inhibit the efficacy of ionizing radiation to inactivate microorganisms in water due to reactions between different solutes in water and hydroxyl radicals (Hoigne, 1997; Sommer et al., 2001; Khan et al., 2017b).

Alkalinity is a primary parameter of water quality and usually expressed as equivalent of calcium carbonate ($CaCO_3$, WHO, 2011). The alkalinity of water affects the presence of inorganic ions, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) and is mainly in the form of bicarbonate (93 %) at the pH around neutrality (Wang et al., 2016). Nitrate, another inorganic ion, is commonly detected in surface water and groundwater (Wang and Chu, 2016) in fresh-cut produce wash water (Weng et al., 2016). Nitrate does

not react with hydroxyl radical but reacts with hydrated electrons (e_{aq}^-) and hydrogen radicals (˙H) (Khan et al., 2017b). Dissolved organic carbon (DOC) is naturally presented in water and defined as the fraction of organic matter that passes through a 0.45 µm filter (Chahal et al., 2016; Wojnarovits and Takacs, 2017). Humic substances (fulvic and humic acids) are the main DOC fractions in freshwater (Westerhoff et al., 2007). The standard samples from the Suwannee River acquired from the International Humic Substances Society are usually used in scientific investigations as a surrogate of DOC (Wang et al., 2016; Wojnarovits and Takacs, 2017).

Hydrogen peroxide (H_2O_2) has been studied extensively in water disinfection (Batterman et al., 2000; Qin et al., 2017; Wang et al. 2019) and fresh produce sanitization treatments to eliminate microorganisms (Olaimat and Holley, 2012; Ramos et al., 2013; Trzaskowska et al., 2018; Hong et al., 2019). Hydrogen peroxide recognized as a safe chemical sanitizer, has bacteriostatic and bactericidal activity because of its characteristics as an oxidant and its capacity to produce other cytotoxic oxidizing species such as hydroxyl radicals (Rico et al., 2007; Olmez and Kretzschmar, 2009; USFDA, 2014). This chemical compound is environmentally friendly and quickly decomposes into water, it is colorless, and non-corrosive (Fallik, 2014; Meireles et al., 2016).

Due to its negative effect on overall quality, low concentration (1-2 %) of H_2O_2 has been recommended for fresh produce applications (Ramos et al., 2013; USFDA, 2014). On the other hand, when applied alone, the efficacy of low concentration H_2O_2 to reduce the pathogenic bacterial counts on the fresh produce is limited (Huang and Chen, 2011) and not adequate to avoid the cross contamination in the vegetables washing water

(Van Hauta et al., 2015). Therefore, several studies have suggested the use of hydrogen peroxide combined with organic acids (Venkitanaryanan et al., 2002; Huang and Chen, 2011), ultraviolet (UV) light, (Guo et al., 2017; Huang et al., 2018b), titanium dioxide (TiO_2) (Foster et al., 2011; Kim et al., 2013), commercial metal ions (Van Haute et al., 2015), and mild heat treatment (Lin et al., 2002) to increase its antimicrobial efficacy and achieve a more effective disinfection process.

There is no available information about the combined use of electron beam irradiation with H_2O_2 for water disinfection in the fresh produce industry. Therefore, the aim of the present study was to (1) characterize and quantify the effect of water quality parameters (alkalinity, nitrate ions, dissolved organic carbon, and pH) and hydrogen peroxide on the efficacy of electron beam irradiation against the inactivation of *S.* Typhimurium ATCC strain 13311 in aqueous solutions and, (2) obtain predictive models to quantify the inactivation kinetics of the pathogen in aqueous solutions.

4.3 Materials and methods

4.3.1 Bacterial culture

Salmonella enterica subsp. *enterica* serotype Typhimurium ATCC strain 13311 (hereafter called *S.* Typhimurium) was provided from Dr. Castillo`s Food Microbiology Laboratory (Department of Animal Science, Texas A&M University). Frozen stocks were maintained at -80°C. Prior to use, an inoculum was removed from frozen culture with a loop, streaked onto 9 mL Trypticase Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD) and incubated at 37°C for 24 h. Then, single colony isolates was obtained by streaking on Trypticase Soy Agar (TSA; Difco, Becton Dickinson, Sparks, MD) and

incubated at 36 °C for 24 h through two successive transfers on TSA. Colonies were stored on a TSA slant at 5 °C as working cultures and used within 30 days.

4.3.2 Inoculum preparation

In general, the inoculation procedure of Keskinen et al. (2009) was followed with minor modifications. The inoculum was prepared by transferring a loopful of the working stock to 9 mL TSB and incubated at 37°C for 18-24 h. After incubation, each test tube was centrifuged and washed for three consecutive times (3000 x g for 15 min) with sterile deionized (DI) water at 5°C. Each of the cell pellets obtained was resuspended in 9 mL sterilized DI water or sterilized treatment solutions. The average final concentration of the strain of *S.* Typhimurium used in this study was about 10^9 CFU/ml as checked by plate counting on TSA. Before each experiment, fresh cultures were prepared.

4.3.3 Chemicals and preparation of aqueous solutions

Calcium carbonate ($CaCO_3$, EMD chemicals Inc., Gibbstown, NJ, USA), nitrogen-nitrate standard solution (4430 mg/l as NO_3, Hach Company, Loveland, CO, USA), Suwannee River fulvic acid (FA) standard II (SRFA, $C_{14}H_{12}O_8$, International Humic Substance Society, St. Paul, MN, USA), and hydrogen peroxide solution (H_2O_2, 50 wt. %, Honeywell Fluka™, Honeywell International Inc., Muskegon, MI, USA) were used as received without purification.

Stock solutions were prepared in deionized (DI) water with 1.0 mM phosphate buffer (PB) (hereafter called buffer solution). Stock solutions of alkalinity (500 mg-$CaCO_3$/l) and natural organic matter (1000 mg-FA/l) were prepared by dissolving 250

mg $CaCO_3$ and 50 mg FA into 500 ml and 50 ml buffer solution, respectively. A stock solution of 1000 mg/l nitrate was prepared by diluting 113 ml of nitrate standard solution into 387 ml buffer solution. Stock solutions of different (5.5, 7.2, and 8.5) pH were prepared from 10 mM PB by mixing 1000 mM KH_2PO_4 and 1000 mM K_2HPO_4 in enough DI water to make volume 100 ml and adjusted to desired pH with 1 N NaOH and 1 N H_2SO_4 if needed. Prior to each experiment, hydrogen peroxide solutions were prepared by dissolving 6.0, 30, and 120 mg into 100 ml sterile buffer solution. Analysis of H_2O_2 in solution was measured using drop wise titration with a standard Hach hydrogen peroxide test kit (Hach Company, Loveland, CO, USA) based on directions in the manual. All aqueous solutions, except the hydrogen peroxide solutions, were sterilized by autoclave for 15 minutes at 121°C and their pH values were separately measured using a digital pH meter (FE20/EL20, Mettler Toledo™, Columbus, OH, USA). Table 4.1 shows the experimental design regarding the type and concentration levels of the aqueous solutions used in this study.

4.3.4 Sample preparation

Non-sterile cylindrical sample vials (14 mm diameter) x 1 mm height) (Dynalon Labware, Dynalab Corp., Rochester, NY, USA) were initially sterilized using 70 % ethanol and air dried in a biological safety cabinet (Class II Type A2, Labconco Corporation, Kansas City, MO, USA) for an hour (Jadhav et al., 2013). Each sample was then prepared from 150 µl of the bacterial suspension as described in *Section 4.3.2* and placed in the sterilized sample vial for electron beam treatment. To check the sterility of the cylindrical sample vials, three negative control samples were prepared as 150 µl

Table 4.1 Experimental design (type of aqueous solutions tested and concentration levels)

Treatment	Concentration	pH
Baseline (PB)	1.0 mM	7.20[#] (0.05)[*]
PB (Phosphate buffer)	10.0 mM	5.50 (0.01)
	10.0 mM	7.20 (0.10)
	10.0 mM	8.50 (0.01)
NO_3 (Nitrate)	10 mg/l	7.20 (0.14)
	100 mg/l	7.18 (0.05)
	1000 mg/l	7.17 (0.17)
FA (Fulvic acid)	10 mg/l	7.20 (0.06)
	100 mg/l	7.17 (0.15)
	1000 mg/l	7.15 (0.16)
$CaCO_3$ (Calcium carbonate)	50 mg/l	7.20 (0.09)
	200 mg/l	7.21 (0.07)
	500 mg/l	7.24 (0.12)
H_2O_2 (Hydrogen peroxide)	60 mg/l	7.20 (0.06)
	300 mg/l	7.20 (0.07)
	1200 mg/l	7.20 (0.09)

[#]Values are means of three replications (n = 9)
*Standard deviation

sterilized deionized water without bacterial suspension, before each experiment. Sample vials were then sealed with Parafilm (Bemis NA, Neenah, WI, USA) and placed in heat-sealed sterile Whirl-Pak® bags (Whirl-Pak, NASCO, Fort Atkinson, WI). Afterward, samples were transported to the e-beam facility in an insulated cooler with refrigerant packs. At the accelerator facility, samples were allowed to equilibrate to room temperature (~ 22 °C) for 15 min prior to irradiation.

4.3.5 Electron beam (e-beam) irradiation treatment

The irradiation tests were carried out with a 1.35 MeV Van de Graaff accelerator (High Voltage Engineering Corp, Cambridge, MA) located at the Food Safety Engineering lab in the Hobgood building at Texas A&M University. A Farmer ionization chamber (Markus® Ion Chamber, Type 23343, Radiation Design, Inc., Albertville, MN, USA) was used as a primary standard dosimeter for calibration procedure (Kim et al., 2007) and, to determine the hot spot, which is the location of maximum electrons emitted on the plate about 15 cm away from the electron gun. Then, a radiochromic film dosimeter (RF, Far West Technology Inc., Batch 1086, 42.5µm, Goleta, CA, USA) was placed at the hotspot and irradiated with the target dose. After irradiation, a digital radiochromic reader (Model FWT-92D, Far West Technology Inc., Goleta, CA, USA) was used to read the optical density of the RF. The absorbed dose in kilogray (kGy) was linearly correlated to the optical density of the RF and used for further measurement of the dose absorbed by the samples. The dose mapping procedure is described in detail in Chapter III (Section 3.3.5.1).

4.3.6 Bacterial enumeration

The number of surviving *S*. Typhimurium on each irradiated sample was enumerated. Samples not exposed to the electron beam treatment served as controls of the initial microbial load. Samples of 0.1 ml from the original samples and 0.1 ml from serial dilution in 0.1 % of PW were plated in duplicate on TSA incubated at 37°C for 24-48 h. After incubation, visible colonies were enumerated with the use of a magnifier counter (detection limit was 10 CFU/ml). For quality purposes, dilutions with less than 10 colonies (average of 2 plates) were not considered in the calculations (Sutton, 2011).

4.3.7 Microbial inactivation kinetics

4.3.7.1 Primary models

The GlnaFiT inactivation model-fitting tool (Geeraerd et al., 2005) was used to develop the microbial survival curves (CFU/ml vs. dose) for *S.* Typhimurium and calculate the inactivation model parameters for this pathogenic strain. The **shoulder plus log-linear model (LLS)** (Geeraerd et al., 2000) was used in this study due to its great capability to predict microbial death kinetics as proved in Chapter III (Section 3.4.1). The inactivation resistance parameters were calculated using the equation below

$$N_D = N_o e^{-k*SI}\left(\frac{e^{-k*SI}}{1+(e^{-k*SI}-1)e^{-k*D}}\right) \quad [4.2]$$

Where, SI is the shoulder length (kGy).

For comparison purposes, the parameter '*5D*', defined as the dose required to inactivate 99.999% of the microbial population, was used in the process design criteria as recommended for irradiation of fresh produce by Kim and Moorman (2017). The average D_{10} value, $D_{10\text{-}average}$, was calculated using the relationship below (van Asselt and Zwietering, 2006):

$$D_{10-average} = [SI + (5 * D_{10})]/5 \quad [4.3]$$

4.3.7.2 Secondary models

The Davey (linear Arrhenius) model (Davey, 1993) was used to compare the impact of the concentration of nitrate and fulvic acid (FA) on the inactivation rate constant as a function of dose, *k(D)*, calculated from Eq. (4.2) as:

$$\ln(\frac{1}{k(D)}) = A_1 + A_2C + A_3C^2 \quad [4.4]$$

Where, A_1, A_2, and A_3 are regression parameters and *C* is the concentration of solute (mg/l).

4.3.7.3 Kinetic inactivation model evaluation

The coefficient of determination (R^2) and root mean square error (RMSE) were used to determine the goodness of fit of the primary model.

In addition of R^2 and RMSE, the bias, B_f, and accuracy, A_f, factors as presented by Eq. (4.5) and Eq. (4.6), respectively, used to determine the goodness of fit of the secondary models (Ross et al., 2000). The primary aim of these indexes was to enable to comparison between predictions and independent observations (Perez-Rodriguez and Valero, 2013). If $B_f = A_f = 1$, there is a perfect agreement between observed and predicted data (Omac et al., 2018):

$$A_f = 10^{\frac{\sum\left|\log(\frac{P}{O})\right|}{n}} \quad [4.5]$$

$$B_f = 10^{\frac{\sum\log(\frac{P}{O})}{n}} \quad [4.6]$$

Where, *P* and *O* are the predicted and observed values, respectively, and *n* is the number of observations.

Statistical analysis was performed using the SPSS (version 20.0 for windows, 2011). Each parameter calculated from primary models was determined for each treatment and analyzed by an analysis of variance (ANOVA) using Tukey`s multiple range tests. Statistical significance was determined at the $P<0.05$ levels.

4.3.8 Experimental design

Six different sets of experiments regarding water quality parameters and H_2O_2 were conducted (Table 4.1). Aqueous solutions including various pH (5.5, 7.2, and 8.5), nitrate (10, 100, and 1000 mg/l), fulvic acid (10, 100, and 1000 mg/l), calcium carbonate (50, 200, and 500 mg/l), and hydrogen peroxide (6.0, 30.0, and 120 mg/l) were combined with the e-beam treatment to determine their impact on the radiation sensitivity of *S.* Typhimurium ATCC strain 13311. The effect of each compound on the pathogen`s radiation sensitivity in aqueous solutions was quantified using the primary models described in Section 4.3.7.1.

The water quality parameters used in the present study were selected based on literature data (Lehto et al., 2014; Van Haute et al., 2015; Gil et al., 2016; Weng et al., 2016). The reported pH, NO_3, and DOC values of fresh produce washing water ranged from 6.00 to 8.10, 1.92 to 253 mg/l, and 9.9 to 690 mg/l, respectively. Fulvic acid was used as DOC surrogate in the present study because its average molecular weight is less than 1000 Da (Wang et al., 2016), so it can easily diffuse into the cytoplasm (Decad and Nikaido, 1976). The ninety percent confidence interval for water hardness as $CaCO_3$ in the U.S. ranged from 30.3 mg/l to 370 mg/l, with a median value of 162 mg/l (DeSimone, 2009).

Three samples were prepared for each point of target dose (0.15, 0.30, 0.45, 0.60, and 0.75 kGy) for each solution before the e-beam irradiation treatment. Three replications were done for each experiment.

4.4 Results and discussion

4.4.1 The effect of water quality parameters on radiation sensitivity of S. Typhimurium

Figures 4.1 through 4.4 display the measured and predicted survival curves of *S.* Typhimurium ATCC strain 13311 irradiated in various aqueous solutions. The curves obtained for all aqueous solutions yielded a high correlation coefficient ($R^2 > 0.988$) and low root mean square error (RMSE < 0.316) (Table 4.2 and 4.3), indicating the goodness of fit of the LLS model.

The $D_{10\text{-average}}$ values for *S.* Typhimurium irradiated in DI water with 10 mM PB by e-beams were 0.155±0.002 kGy, 0.156±0.002 kGy, and 0.152±0.006 kGy when the pH changed as 5.5 (acidic), 7.2 (neutral), and 8.5 (alkaline), respectively, (Figure 4.1). As shown in Table 4.2, the pH did not affect ($P > 0.05$) the shoulder length (*SI*) of survival curves. The *SI* values increased ($P > 0.05$) by 95.69 % and 86.27 % at pH 5.5 and 8.5 respectively, compared with that at neutral conditions of pH 7.2 (Table 4.2). The effect of pH on the *SI* values may be simply related to the fact that under acidic conditions, hydrated electrons (e_{aq}^-) were scavenged by hydrogen ions (H^+) and converted to hydrogen atoms (Eq. (4.7)) while under alkaline condition, hydrogen ions react with hydroxyl radicals to produce hydrated electrons (Eq. (4.8)) (Sayed et al., 2016). Thus, more hydrogen ions and hydrated electrons exist which could scavenge

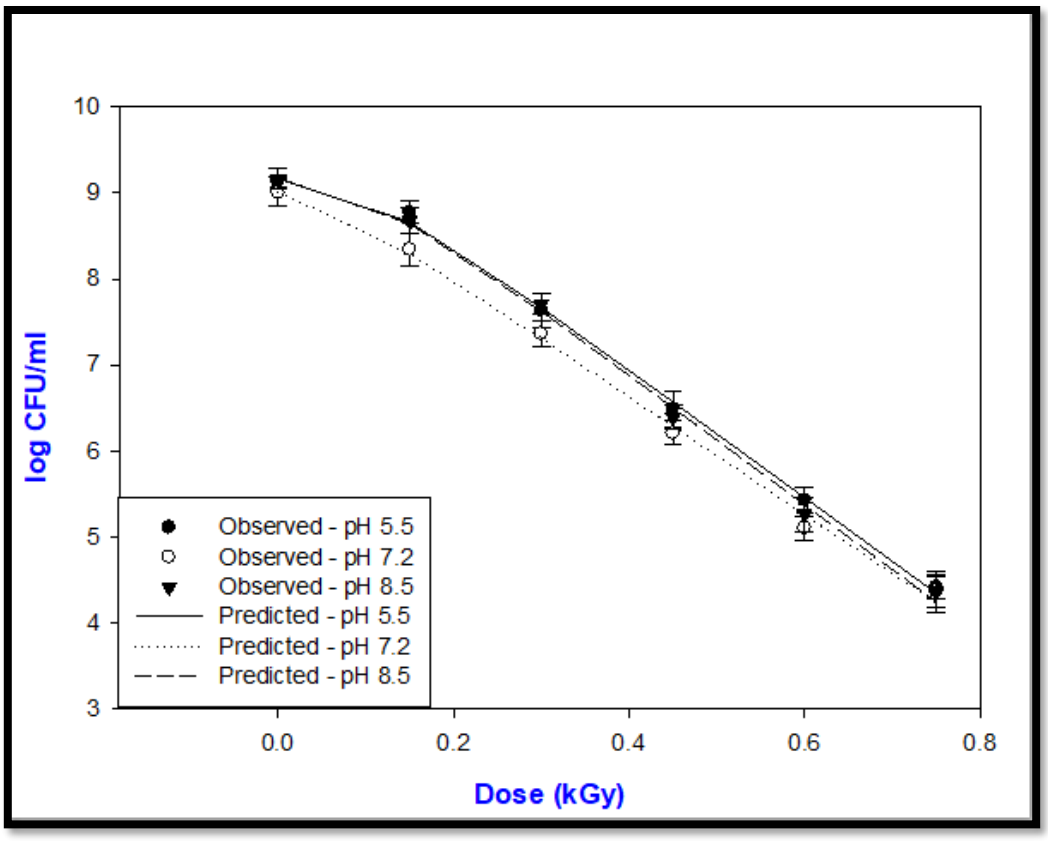

Figure 4.1 Survival curves for *S.* Typhimurium ATCC strain 13311 in 10.0 mM solution at different pH values to electron beam irradiation and fitted by the LLS (log-linear plus shoulder) model (Eq. (4.2)).

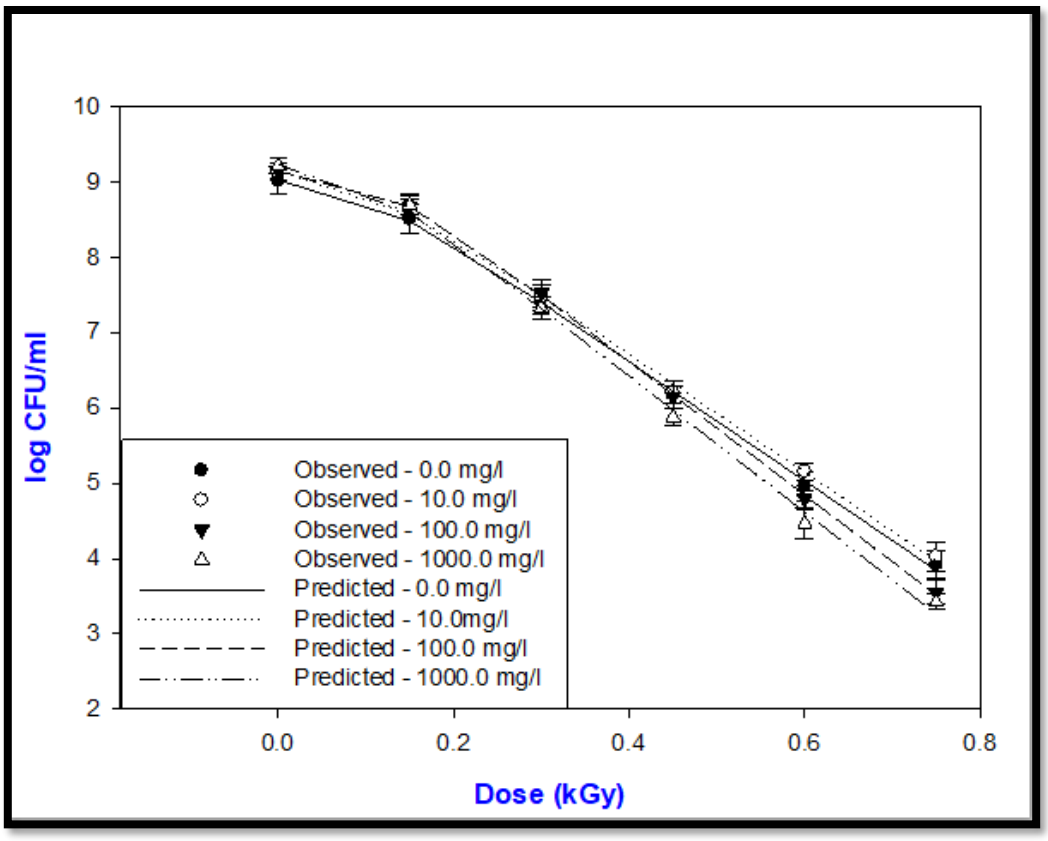

Figure 4.2 Survival curves for *S.* Typhimurium ATCC strain 13311 in various concentrations of NO_3 (nitrate) aqueous solution exposed to electron beam irradiation. Continuous lines represent predicted values from the LLS (log-linear plus shoulder) model (Eq. (4.2))

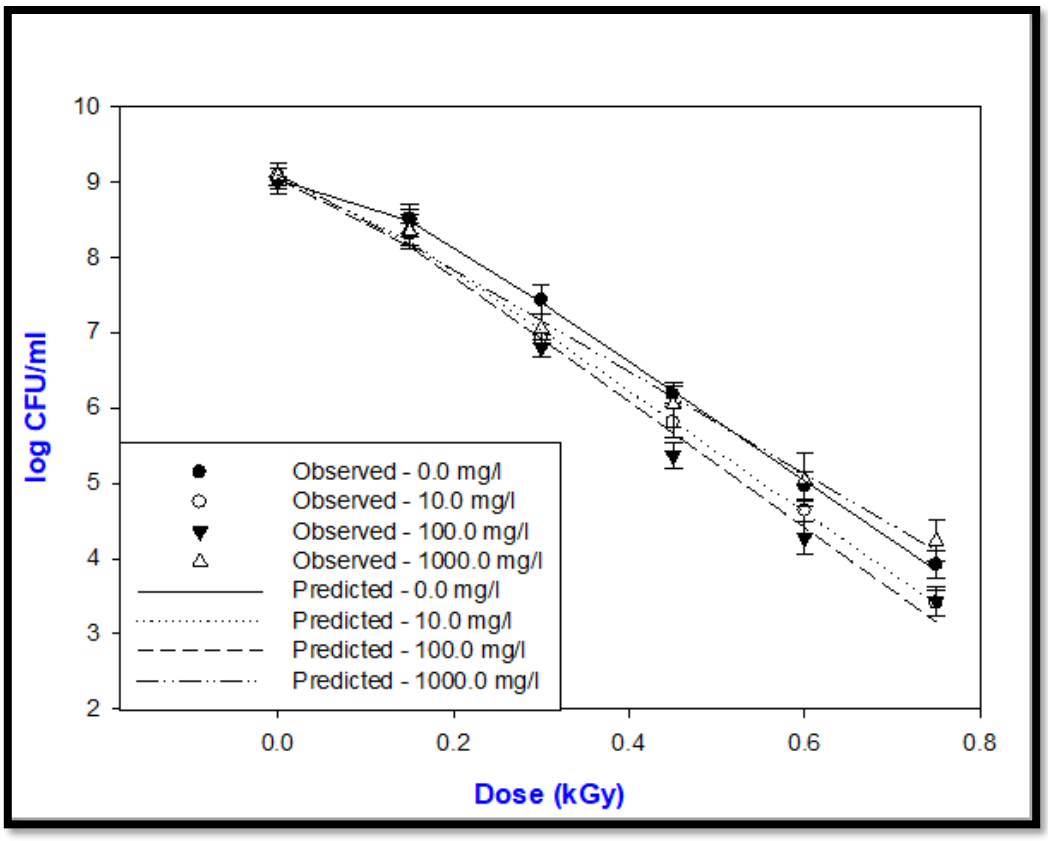

Figure 4.3 Survival curves for *S.* Typhimurium ATCC strain 13311 in various concentration of FA (fulvic acid) aqueous solutions exposed to electron beam irradiation. Continuous lines represent predicted values from the LLS (log-linear plus shoulder) model (Eq. (4.2))

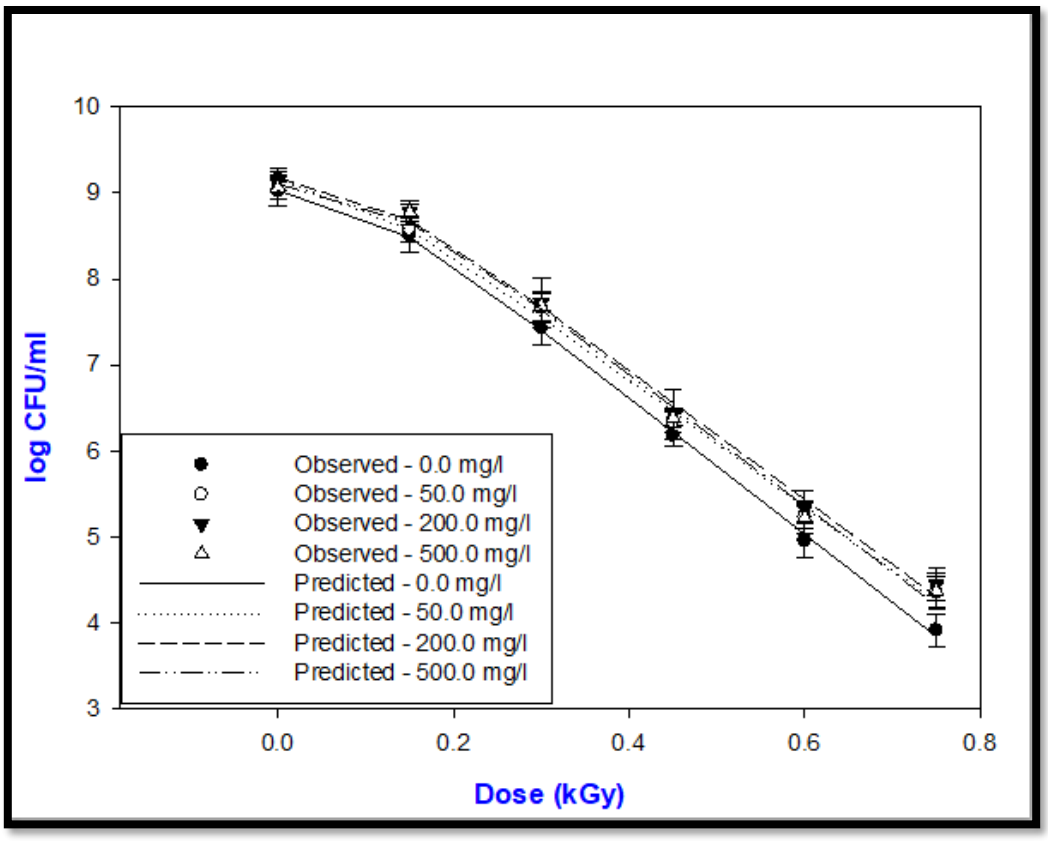

Figure 4.4 Survival curves for *S.* Typhimurium ATCC strain 13311 in various concentration of $CaCO_3$ (calcium carbonate) aqueous solutions exposed to electron beam irradiation. Continuous lines represent predicted values from the LLS (log-linear plus shoulder) model (Eq. (4.2))

Table 4.2 Survival kinetics parameters obtained after fitted by the LLS (log-linear plus shoulder) model (Eq. (4.2)) for *S.* Typhimurium ATCC strain 13311 in different aqueous solutions

Treatment	**Value**	**[a]N_o (log CFU/ml)**	**[b]SI (kGy)**	**[c]k (kGy^{-1})**	**$D_{10\text{-average}}$ (Eq. (4.3)) (kGy)**	**[d]RMSE**	**[e]R^2**
pH (10.0 mM Phosphate buffer)	5.5	$_w$9.16 (0.04)*	$_w$0.100 (0.028)	$_w$17.07 (0.58)	$_w$0.155 (0.002)	0.094	0.998
	7.2	$_w$9.01 (0.14)	$_w$0.051 (0.026)	$_w$15.77 (0.61)	$_w$0.156 (0.002)	0.137	0.997
	8.5	$_w$9.17 (0.14)	$_w$0.095 (0.011)	$_w$17.32 (0.63)	$_w$0.152 (0.006)	0.125	0.997

*Standard deviation

w: Means within a column, which are not followed by a common subscript letter, are significantly different ($P < 0.05$)

[a]N_o: initial concentration; [b]SI: shoulder length (kGy); [c]k: rate constant (kGy^{-1}); [d]RMSE: root mean square; [e]R^2: coefficient of determination

Table 4.3 Survival kinetics parameters obtained after fitted by the LLS (log-linear plus shoulder) model (Eq. (4.2)) for *S.* Typhimurium ATCC strain 13311 in different aqueous solutions.

[1]Treatment	Concentration (mg/l)	[a]N_0 (log CFU/ml)	[b]SI (kGy)	[c]k (kGy^{-1})	$D_{10\text{-average}}$ (Eq. (4.3)) (kGy)	[d]RMSE	[e]R^2
Baseline		$_{w}$9.03 (0.20)*	$_{w}$0.095 (0.051)	$_{y, w}$18.33 (1.59)	$_{y, w}$0.145 (0.003)	0.071	0.999
NO_3	10	$_{w}$9.15 (0.04)	$_{w}$0.090 (0.08)	$_{z, x, w}$18.06 (0.37)	$_{y, w}$0.146 (0.002)	0.101	0.998
	100	$_{w}$9.15 (0.05)	$_{w}$0.114 (0.008)	$_{y, x}$20.41 (0.26)	$_{y, x}$0.136 (0.003)	0.043	1.000
	1000	$_{w}$9.24 (0.05)	$_{w}$0.088 (0.024)	$_{y}$20.80 (0.76)	$_{y}$0.128 (0.002)	0.152	0.997
FA	10	$_{w}$9.08 (0.07)	$_{w, x}$0.046 (0.005)	$_{y, x, w}$18.60 (0.89)	$_{x, y, z}$0.133 (0.007)	0.089	0.999
	100	$_{w}$9.05 (0.02)	$_{w, x}$0.046 (0.014)	$_{x, y, w}$19.26 (0.12)	$_{x, y, z}$0.129 (0.003)	0.316	0.988
	1000	$_{w}$9.10 (0.17)	$_{x}$0.015 (0.007)	$_{z}$15.65 (0.63)	$_{w}$0.150 (0.005)	0.160	0.996
$CaCO_3$	50	$_{w}$9.16 (0.08)	$_{w, x}$0.082 (0.036)	$_{z, w}$17.00 (1.07)	$_{w}$0.152 (0.003)	0.134	0.997
	200	$_{w}$9.17 (0.07)	$_{w}$0.104 (0.026)	$_{z, w}$17.34 (0.07)	$_{w}$0.154 (0.005)	0.142	0.997
	500	$_{w}$9.11 (0.13)	$_{w}$0.110 (0.031)	$_{z, w}$17.66 (1.16)	$_{w}$0.153 (0.003)	0.159	0.996

*Standard deviation

w,x,y,z: Means within a column, which are not followed by a common subscript letter, are significantly different ($P < 0.05$)

[1]Baseline: 1.0 mM phosphate buffer; NO_3: nitrate; FA: fulvic acid; $CaCO_3$: calcium carbonate

[a]N_o: initial concentration; [b]SI: shoulder length (kGy); [c]k: rate constant (kGy^{-1}); [d]RMSE: root mean square; [e]R^2: coefficient of determination

hydroxyl radicals according to Eq. (4.9) and Eq. (4.10) respectively. This decrease of hydroxyl radical concentration may increase (P > 0.05) the SI values.

$$e_{aq}^{-} + H^{+} \longrightarrow {}^{\cdot}H \qquad k_7 = 2.3 \times 10^{10}\ M^{-1}s^{-1} \qquad [4.7]$$

$${}^{\cdot}H + OH^{-} \longrightarrow e_{aq}^{-} + H_2O \qquad k_8 = 2.2 \times 10^{7}\ M^{-1}s^{-1} \qquad [4.8]$$

$${}^{\cdot}OH + e_{aq}^{-} \longrightarrow OH^{-} \qquad k_9 = 3 \times 10^{10}\ M^{-1}s^{-1} \qquad [4.9]$$

$${}^{\cdot}OH + {}^{\cdot}H \rightarrow H_2O \qquad k_{10} = 7.0 \times 10^{9}\ M^{-1}s^{-1} \qquad [4.10]$$

The irradiation inactivation rate constant values, *k*, increased (P > 0.05) by 8.26 % and 9.83 % at low pH and increased at 8.5, respectively (Table 4.2). These results make sense because the acid-base equilibrium between superoxide radicals (O^{-}_2) and hydroperoxyl radicals (HO_2^{-}) was largely on the superoxide radicals' side at pH 5.5 in the presence of air or oxygen whereas increasing pH to 8.5 would shift the bicarbonate/ carbonate equilibrium towards carbonate radicals (Wang et al., 2019). Both, superoxide and carbonate radicals can induce DNA damage (van Sonntag, 2006) and increasing concentration of superoxide and carbonate ions increased the *k* value of *S*. Typhimurium in aqueous solution. *S*. Typhimurium grows optimally between pH 6.5 and 7.5 and is capable of growth at pH between 3.99 and 9.5 (Li et al., 2013). The acid tolerance response (ATR) system triggered in *Salmonella* species at the external pH values between 6.0 to 5.5 protects cells from more severe acid stress (Foster and Hall, 1991). Because of this system, *S*. Typhimurium induces the expression of at least 52 acid shock proteins which protect the cell against acid and perhaps other environmental stresses such as reactive oxygen species (ROS, Leyer and Johnson, 1993). Under alkaline conditions, the proton motive force and the protein profile of the outer membrane in

gram-negative including *S*. Typhimurium can be disrupted and altered, respectively, (Wesche et al. 2009). Thus, these proteins may be too sensitive to radiodamage and lose their functions in the cell and outer membrane of the cell which increase the estimated *k* values of *S*. Typhimurium at pH 5.5 and 8.5 compared to their value at pH 7.2. Nevertheless, these changes in *k* values for *S*. Typhimurium under acidic, neutral, and alkaline conditions were not sufficient to increase the decontamination effectiveness of e-beam treatment.

The effect of concentration of nitrate (10, 100, and 1000 mg/l) on the radiation sensitivity of *S*. Typhimurium in buffer solution are presented on Figure 4.2. The addition of 10 mg/l nitrate into buffer solution did not affect ($P > 0.05$) the inactivation parameters (*SI* and *k* values) of the pathogen (Table 4.3). This is probably due to the fact that the radical scavenging efficiency was less than 5.3×10^{-6} s^{-1}, reported as the minimum response level for the dissolved compound to be able to compete with the reaction in Eq. (4.11) (Stefan, 2018).

$$e_{aq}^{-} + O_2 \longrightarrow O_2^{-} \qquad k_{12}=1.9 \times 10^{10}\ M^{-1}s^{-1} \qquad [4.11]$$

Increasing the concentration of nitrate to 100 and 1000 mg/l did not affect ($P > 0.05$) the *SI* values but the *k* values increased ($P < 0.05$) by 11.34 % and 13.49 % (Table 4.3). In general, the calculated $D_{10\text{-}average}$ value of *S*. Typhimurium in buffer solution decreased ($P < 0.05$) by 6.60 % and 11.66 % with the addition of 100 and 1000 mg/l nitrate, respectively. These results prove that the yield of hydroxyl radicals increased in the presence of hydrated electron scavengers in aqueous solutions because hydrated electrons produced by radiolysis of water reacted with nitrate (Eq. (4.12)) instead of with

hydroxyl radicals (Eq. (4.9), Wang et al., 2019). Similarly, Samuni and Czapski (1978) found that the radiation sensitivity of *E. coli* B irradiated in 50 mM phosphate buffer by gamma irradiation increased by 94.12 % in the presence of nitrous oxide (N_2O) and oxygen compared to in the presence of oxygen alone. Furthermore, the results obtained at the present study indicate that the contribution of hydrated electrons on inactivation of *S.* Typhimurium was negligible as reported previously (Sanner and Pihl, 1969). Our findings confirm that the hydroxyl radicals among the water radicals were responsible for the majority indirect damage to cells and are in agreement with *E. coli* B study (Samuni and Czapski, 1978).

$$NO_3^- + e_{aq}^- \rightarrow NO_3^{2-} \qquad k_{11} = 9.7 \times 10^9\ M^{-1}s^{-1} \qquad [4.12]$$

Addition of fulvic acid (FA) affected the radioresistance of S. Typhimurium in phosphate buffer solution (Figure 3). FA at 10 mg/l and 100 mg/l concentrations in buffer solution decreased ($P > 0.05$) SI values by 51.58 % but increased ($P > 0.05$) the *k* values by 1.47 % and 5.07 %, respectively, (Table 4.3). The calculated $D_{10\text{-average}}$ values for the pathogen decreased ($P < 0.05$) by 8.28 % and 11.03 % when 10 mg/l and 100 mg/l FA were added into buffer solution, respectively. These results suggest that when the concentration of FA is 100 mg/l or less, the radiation sensitivity of *S.* Typhimurium in buffer solution increases due to the formation of peroxyl radicals ($^{\cdot}RO_2$) in the presence of air. Likewise, Johansen and Howard-Flanders (1965) pointed out that the formation of peroxyl radicals increased the probability of cell death.

Now, the addition of 1000 mg/l (Table 4.3) decreased ($P < 0.05$) the *SI* and *k* values of *S.* Typhimurium in buffer solution by 84.21 % and 14.62 %, respectively,

while the calculated D1-0-average value of the pathogen increased ($P > 0.05$) by 3.45 % (Table 4.3). This result could be due to the fact that the available oxygen in aqueous solution would mostly react with hydrated electrons (Eq. (4.11)) and hydrogen ions (Eq. (4.13)) formed from the radiolysis of water due to high reaction rate constants, so limited amount of FA was consumed by oxygen and the rest of FA would react with hydroxyl radicals to begin to protect *S*. Typhimurium cells in aqueous solution (Ewing and Kubala, 1987; Matilainen and Sillanpaa, 2010).

$$^{\cdot}H + O_2 \longrightarrow HO_2^{\cdot} \qquad K_{13}=1.2 \times 10^{10}\ M^{-1}s^{-1} \qquad [4.13]$$

Alkalinity is one of the main water quality parameters and the effect of various concentrations (50, 200, and 500 mg/l) of calcium carbonate on the survival of *S*. Typhimurium cells is shown in Figure 4.4. The *SI* and *k* values were not affected ($P > 0.05$) by the added carbonate alkalinity. Therefore, the $D_{10\text{-}average}$ values barely changed ($P > 0.05$) with carbonate alkalinity (Table 4.3). This result was expected, because bicarbonate (HCO_3^-) is well-known as a scavenger of hydroxyl radicals (Eq. (4.14), Wang and Chu, 2016), it does not affect the radiation sensitivity of the studied pathogen because the hydroxyl radical scavenging efficiency was much lower than ~$1.5 \times 10^7\ s^{-1}$, reported as the minimum response level for the additives to begin to protect *E. coli* B/r cells in air saturated aqueous solution against hydroxyl radicals (Ewing and Kubala, 1987). Accordingly, Sommer et al. (2001) found that 159 mg/l of bicarbonate in tap water did not affect the radiation sensitivity of bacteriophages irradiated in water by gamma irradiation because its radical scavenging efficiency is $2.55 \times 10^4\ s^{-1}$.

$$HCO_3^- + {}^{\cdot}OH \rightarrow CO_3^{\cdot-} + H_2O \qquad k_{14} = 8.5 \times 10^6\ M^{-1}s^{-1} \qquad [4.14]$$

In summary, these results indicate that unlike pH and alkalinity, two (nitrate and DOC) of water quality parameters played a role on the effectiveness of e-beam treatment to eliminate *S.* Typhimurium in aqueous solutions. The presence of nitrate (100 mg/l and over) and DOC (100 mg/l and less) affect ($P < 0.05$) e-beam inactivation efficiency due to their effect on the yield of hydroxyl radicals formed by radiolysis of water. Our results regarding the presence of nitrate demonstrate that the concentration of dissolved oxygen in aqueous solution was not adequate to react with all these hydrated electrons from radiolysis water. Therefore, the addition of oxygen or a hydrated electron scavenger is required to enhance the inactivation effectiveness of e-beam treatment in aqueous solution.

4.4.2 The effect of hydrogen peroxide (H_2O_2) on radiation sensitivity of S. Typhimurium

Survival curves obtained at various concentration of H_2O_2 were fitted by the primary model (Eq. 4.2) to estimate the inactivation kinetic parameters. The determination coefficients (R^2) and root mean square error (RMSE) were used to evaluate the goodness of it (Table 4.4). The R^2 values was higher than 0.988, which means that less than 1.2 % of total response variation remained unexplained by the log-linear plus shoulder (LLS) model. The RMSE values ranged from 0.071 to 0.350 and can be assumed close to the experimental data (Zeng et al., 2014).

The reductions (log CFU/ml) of *S.* Typhimurium irradiated in various hydrogen peroxide aqueous solutions using e-beam irradiation are depicted in Figure 4.5. The

Table 4.4 Survival kinetics parameters obtained after fitted by the LLS (log-linear plus shoulder) model (Eq. (4.2)) for *S.* Typhimurium ATCC strain 13311 in different aqueous solutions.

[1]Treatment	Concentration (mg/l)	[a]N_o (log CFU/ml)	[b]SI (kGy)	[c]k (kGy^{-1})	$D_{10\text{-average}}$ (Eq. (3)) (kGy)	[d]RMSE	[e]R^2
Baseline		$_w$9.03 (0.20)*	$_w$0.095 (0.051)	$_w$18.33 (1.59)	$_w$0.145 (0.003)	0.071	0.999
H_2O_2	60	$_w$9.06 (0.15)	$_w$0.122 (0.004)	$_x$29.66 (0.46)	$_x$0.102 (0.002)	0.208	0.996
	300	$_w$9.06 (0.01)	$_w$0.114 (0.026)	$_x$29.21 (1.56)	$_x$0.102 (0.001)	0.200	0.996
	1200	$_w$9.01 (0.08)	$_w$0.102 (0.020)	$_x$28.12 (1.62)	$_x$0.102 (0.001)	0.350	0.988

*Standard deviation

w,x,y,z: Means within a column, which are not followed by a common subscript letter, are significantly different ($P < 0.05$)

[1]Baseline: 1.0 mM phosphate buffer; H_2O_2: hydrogen peroxide

[a]N_o: initial concentration; [b]SI: shoulder length (kGy); [c]k: rate constant (kGy^{-1}); [d]RMSE: root mean square; [e]R^2: coefficient of determination

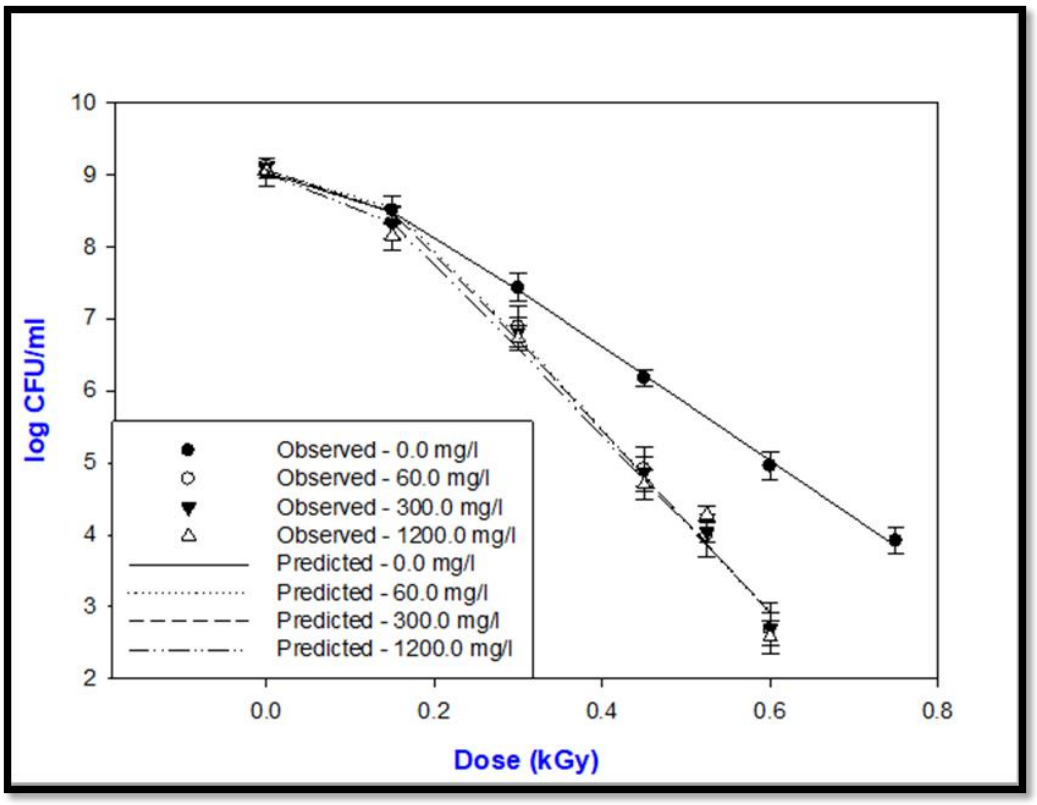

Figure 4.5 Survival curves for *S*. Typhimurium ATCC strain 13311 in various concentration of H_2O_2 (hydrogen peroxide) aqueous solutions exposed to electron beam irradiation. Continuous lines represent predicted values from the LLS (log-linear plus shoulder) model (Eq. (4.2))

number of CFU/ml was reduced ($P < 0.05$) by over 6.40 log when H_2O_2 was added. The *SI* values of *S.* Typhimurium in buffer solution increased ($P > 0.05$) by 28.42 %, 20.0 %, and 7.37 % with the addition of 60, 300, and 1200 mg/l H_2O_2, respectively, compared to the values in the absence of H_2O_2 (Table 4.4). Similarly, the *k* values increased ($P < 0.05$) by 61.81 %, 59.36 %, and 53.41 % when 60, 300, and 1200 mg/l H_2O_2, respectively, were added into buffer solution with no H_2O_2 (Table 4.4). As expected, the estimated $D_{10\text{-}average}$ of this pathogen in buffer solution decreased ($P < 0.05$) by 29.66 % with the addition of different H_2O_2 concentrations (Table 4.4). This finding confirms that the presence of H_2O_2 increases the sensitivity of *S.* Typhimurium to e-beam irradiation. The inactivation effect of combined e-beam irradiation and H_2O_2 treatment may be explained in two ways. First, H_2O_2 had a direct oxidative action on cells of *S.* Typhimurium rendering them weaker and more sensitive to the direct or indirect effect of e-beam irradiation, since H_2O_2 could cause damage to DNA, RNA, protein, and lipids (Farr and Kogoma, 1991). Second, the residual H_2O_2 in buffer solution reacted with hydrated electron and hydrogen atom as illustrated by Eq. (4.15) and Eq. (4.16) (Emmi et al., 2012), respectively, and produced hydroxyl radicals thus increasing the radiation sensitivity of *S.* Typhimurium cells.

$$e_{aq}^{-} + H_2O \rightarrow {}^{\cdot}OH + OH^{-} \qquad k_{15} = 1.2 \times 10^{10}\ M^{-1}s^{-1} \qquad [4.15]$$

$$H + H_2O_2 \rightarrow {}^{\cdot}OH + H_2O \qquad k_{16} = 3.47 \times 10^{7}\ M^{-1}s^{-1} \qquad [4.16]$$

The inactivation parameters (*SI* and *k* values) of *S.* Typhimurium in aqueous solutions slightly decreases ($P > 0.05$) when the hydrogen peroxide concentration in buffer solution increased (Table 4.4). This result may be explained as in response to an

increased flux of H_2O_2, where the cellular concentration of at least 30 proteins becomes raised over the basal levels (Farr and Kogoma, 1991). Thus, these proteins increase the concentration of dissolved organic carbon (DOC), which reacts with dissolved oxygen resulting in formation of peroxyl radicals causing lethal damage to *S.* Typhimurium cells and decrease the *SI* and *k* values as observed in the case of FA in the present study. Overall, the increased H_2O_2 concentration did not have a significant ($P > 0.05$) impact on the $D_{10\text{-}average}$ values of the pathogen (Table 4.4). This result is explained by the reduced concentration of H_2O_2 in the samples, down to below the detection limit (0.2 mg/l) before e-beam irradiation treatment; mainly because of the degradation of H_2O_2 by enzymatic activity including catalase and superoxide dismutase (Kim and Thayer, 1995).

Emmi et al. (2012) found that the yield of hydroxyl radical increased asymptotically as concentration of H_2O_2 grows, reaching the maximum of 0.52 ± 0.02 µmol/J at H_2O_2 concentration, which was between 170 and 340 mg/l in air saturated water. Similarly, Liu et al. (2016) found that when absorbed dose was 1.0 kGy, the degradation efficacy of carbamazepine (CBZ) in aqueous solution using e-beam treatment increased with the addition of 340 mg/l H_2O_2 but it decreased with the addition of 3401 mg/l and over H_2O_2 because hydroxy radical reacts with the excess H_2O_2. Thus, the slightly change in *k* values (Table 4.4) can be explained by the difference in residual concentration of H_2O_2 which means the effectiveness of e-beam treatment could be still increased with the addition of H_2O_2 to inactivate *S.* Typhimurium in aqueous solution. However, a similar trend was not obviously seen in the present study because the concentration of H_2O_2 decreased to below detection limit due to the time (45 min)

needed to transport samples from the laboratory, where samples were prepared, to the e-beam facility.

4.4.3 Secondary models for inactivation of S. Typhimurium in various aqueous solutions

The understanding of the effect of environmental parameters on the inactivation of microorganisms in foods is crucial for the development, as well as for realistic, use of predictive microbiology models (McKellar and Lu, 2004). Baranyi et al. (1999) pointed out that secondary models not including all environmental parameters substantial in food were to be incomplete and require expansion to provide their effect on microbial kinetics. As mentioned in Section 4.3.8, these water quality parameters are already present in fresh produce wash water. The results from the primary models demonstrate that the presence of nitrate and fulvic acid (FA) significantly affected the microbial kinetics. The relationship between the inactivation rate constant and concentration of nitrate (Eq. (4.17)) and FA (Eq. (4.18)) for *S.* Typhimurium, respectively, were:

$$k(NO_3) = \frac{1}{e^{(-2.91-0.0005*C+6.83*10^{-7}*C^2)}} \quad [4.17]$$

$$k(FA) = \frac{1}{e^{(-2.90-0.0013*C+1.17*10^{-6}*C^2)}} \quad [4.18]$$

The effect of concentration of nitrate and fulvic acid on the inactivation rate constant as a function of dose (*k(D)*) estimated from the log-linear plus shoulder (LLS) model (Eq. (4.2)) for *S.* Typhimurium in aqueous solutions is illustrated in Figure (4.6). The parameters and mathematical evaluation indexes of the secondary models are displayed in Table 4.5. Determination coefficient (R^2) and root mean square error (RMSE) values of the fits varied from 0.998 to 0.975 and from 0.007 to 0.020,

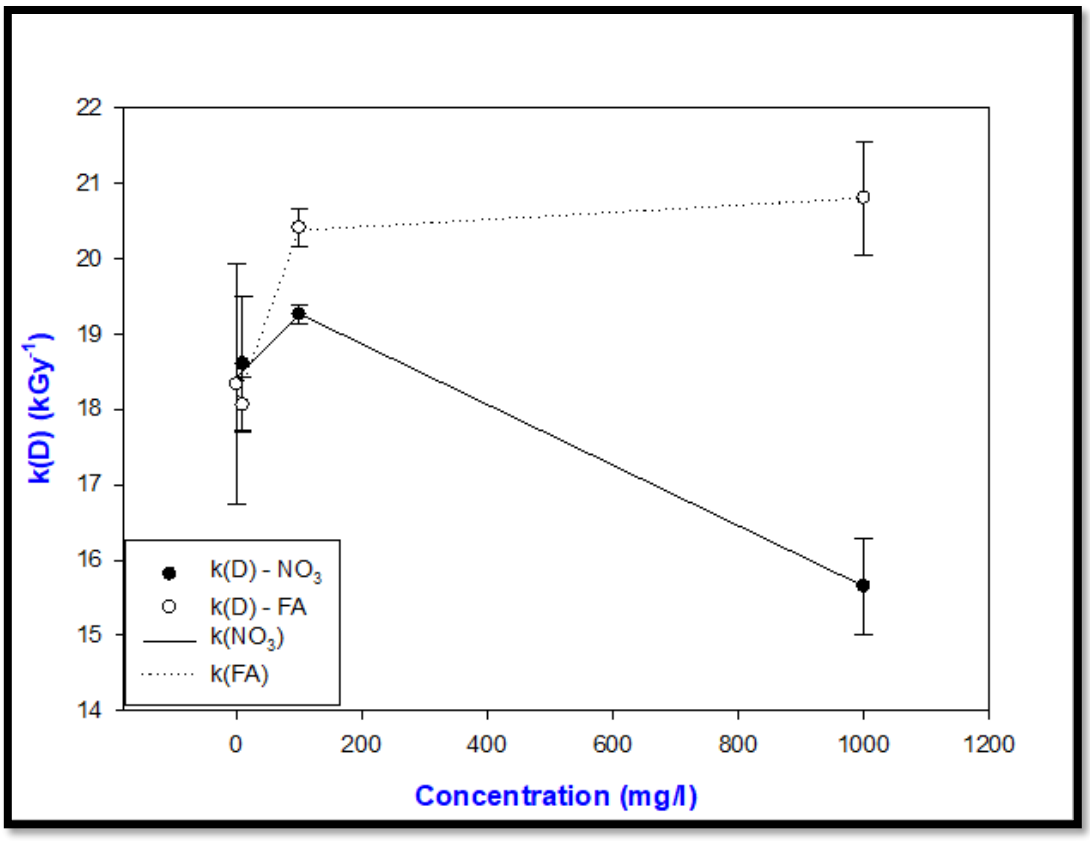

Figure 4.6 Observed (k(D)) and predicted (k(FA)) inactivation rate constant for *S.* Typhimurium ATCC strain 13311 in an aqueous solution as a function of the concentration of nitrate (NO_3) and fulvic acid (FA).

Table 4.5 Coefficients of Eq. (4.4) used to estimate the values of inactivation rate constant (k(D)) obtained from the fit of log-linear plus shoulder model (LLS; Eq. (4.2)) as a function of the concentration of chemical agent in 1.0 mM phosphate buffer (PB) for *S.* Typhimurium ATCC strain 13311 in aqueous solutions

Parameter /Treatment	**[a]A_1**	**95% CI**	**[b]A_2**	**95% CI**	**[c]A_3**	**95% CI**	**[d]R^2**	**[e]RMSE**	**A_f (Eq. (5))**	**B_f (Eq. (6))**
[1]NO_3	-2.91	(-2.98, -2.85)	-0.0005	(-0.0016, 0.0007)	6.83E-07	(-5.14E-07, 1.88E-06)	0.998	0.007	1.00	1.00
[2]FA	-2.90	(-3.09, -2.71)	-0.0013	(-0.0049, 0.0023)	1.17E-06	(-2.35E-06, 4.68E-06))	0.975	0.020	1.00	1.00

[1]NO_3: nitrate; [2]FA: fulvic acid

[a,b,c]A_1, A_2, and A_3, regression parameters of Eq. (4)

[d]R^2: coefficient of determination

[b]RMSE: root mean square error

respectively, confirming that Eq. (4.4) can describe the effect of concentration of nitrate and fulvic acid on the inactivation rate constant, *k*, of the pathogen with good accuracy. In addition, A_f and B_f factors were 1.00 for both models indicating perfect agreement between the inactivation rate constant values observed and the ones predicted (Baranyi et al., 1999).

It appears the inactivation rate constant of *S.* Typhimurium in aqueous solution increased with added nitrate up to (100 mg/l), and then leveled off (Fig. (4.6)). This plateau effect could be a result of the reaction between nitrate and hydrated electrons in equilibrium. This result indicates that the availability of free hydrated electron was not negligible because they react with hydroxyl radicals (˙OH) and protect microbial cells against ˙OH damage. Similarly, the inactivation rate constant first increases with increasing FA dose up to 100 mg/l, and then starts to decrease. This result can be due to fact that FA compete with oxygen for peroxyl radical and interfere in the formation of this radical when the concentration of FA over 100 mg/l. Accordingly, Johansen and Howard-Flanders (1965) found that when the concentration of mercaptoethanol was two and three hundred as high as the concentration of oxygen, the radiation sensitivity of *E. coli* B/r irradiated in buffer saline by X-rays reduced to halfway of maximal value because of competition of mercaptoethanol and oxygen reacting with peroxyl radical. Likewise, Selma et al. (2008) reported that higher decontamination efficiency of heterogeneous photocatalytic disinfection was observed in carrot wash water, where turbidity and organic matter were lower than those in the wash water for lettuce,

escarole, chicory, onion, and spinach due to the reaction between hydroxyl radicals and organic substances.

4.5 Conclusion

Modifications of initial pH between 5.5 and 8.5 did not influence ($P > 0.05$) the effectiveness of e-beam treatment to inactivate *S.* Typhimurium in deionized water with 10.0 mM phosphate buffer. The inactivation rate constant slightly increased ($P > 0.05$) under the acidic and alkaline conditions compared with the neutral condition because of stress conditions for bacterial cells. This suggests that under the higher acidic and alkaline conditions combined with the e-beam treatment have a possible to enhance the inactivation *S.* Typhimurium in aqueous solution.

The various concentration (50, 200, and 500 mg/l) of $CaCO_3$, representing alkalinity of water, did not ($P > 0.05$) affect the radiation sensitivity of *S.* Typhimurium irradiated in aqueous solution by e-beam treatment. The $D_{10\text{-}average}$ values for this pathogen lightly increased ($P > 0.05$) with the addition of $CaCO_3$ indicating that the alkalinity of fresh produce wash water would not affect the effectiveness of e-beam treatment for inactivation of microorganisms in water.

The effect of inorganic substance (nitrate) naturally presenting in wash water on the radiation sensitivity of *S.* Typhimurium in aqueous solution changed based on the concentration of nitrate used (10, 100, and 1000 mg/l). The addition of 10 mg/l nitrate into buffer solution did not have a significant impact on the radiation sensitivity of the pathogen in aqueous solution but 100 mg/l and 1000 mg/l did it ($P < 0.05$). The estimated $D_{10\text{-}average}$ values decreased ($P < 0.05$) by 6.21 % and 11.72 % with the

addition of 100 and 1000 mg/l nitrate, respectively, compared to the $D_{10\text{-}average}$ value for buffer solution. The effect of nitrate, a hydrated electron scavenger, indicates that the amount of dissolved oxygen in aqueous solution was not adequate to react with all hydrated electrons formed from radiolysis water. Hence, it is recommended to add oxygen or hydrated electron scavengers to increase the effectiveness of e-beam irradiation to inactivation of pathogens in water or fresh produce wash water.

The effect of the organic substance (fulvic acid (FA)) in aqueous *S.* typhimurium suspension on the effectiveness of e-beam treatment was evaluated using different concentrations (10, 100, and 1000 mg/l) of FA. The calculated $D_{10\text{-}average}$ values of the pathogen in buffer solution decreased ($P < 0.05$) by 8.28 % and 11.03 % when 10 mg/l and 100 mg/l of FA were added into buffer solution while the calculated D10-average value of this bacterium did not change ($P > 0.05$) with 1000 mg/l of FA added into buffer solution. It is our recommendation that the concentration of dissolved organic carbon (DOC) in wash water was kept under control during the e-beam treatment to ensure the inactivation of microorganisms.

The e-beam treatment for the inactivation of *S.* Typhimurium in aqueous solution in the absence of and in the presence of hydrogen peroxide (H_2O_2) was compared. In the absence of H_2O_2 condition, the e-beam treatment (dose of 0.75 kGy) decreased the population of *S.* Typhimurium inoculated in buffer solution by 5.10±0.019 log CFU/ml. On the other hand, the combined e-beam treatment (dose of 0.60 kGy) with various concentration (60, 300, and 1200 mg/l) of H_2O_2 decreased the population of *S.* Typhimurium in buffer solution by 6.44±0.031 log CFU/ml regardless of H_2O_2

concentration. The estimated $D_{10\text{-}average}$ values of *S*. Typhimurium irradiated in buffer solution by e-beam irradiation decreased ($P < 0.05$) by 29.66 % when 60, 300, and 1200 mg/l of H_2O_2, used as sanitizing agent in fresh produce industry, were added into this buffer solution. These results suggest that the combined treatments of e-beam and H_2O_2 are an effective alternative to prevent cross contamination via wash water used for fresh produce. This hurdle technology can be applied as postharvest technology to achieve 5-log reductions of pathogenic microorganisms on fresh produce as recommended by Doona et al. (2015).

CHAPTER V

THE EFFICACY OF INTEGRATED TREATMENT OF HYDROGEN PEROXIDE AQUEOUS SOLUTION AND ELECTRON BEAM IRRADIATION ON INACTIVATION OF *SALMONELLA SPP.* ON GRAPE TOMATOES

5.1 Overview

The purpose of this study was to investigate the efficacy of integrated electron beam (e-beam) irradiation and hydrogen peroxide (H_2O_2) aqueous solution treatments to inactivate mixed strains of *Salmonella* inoculated on grape tomatoes. A mixed bacterial cocktail composed of a 5-serotype mixture of *Salmonella enterica* (Rissen SAL1449, Montevideo SAL4599, Saintpaul 476398, Typhimurium ATCC 13311, and Typhimurium ATCC 700720) was applied to whole grape tomatoes by dip inoculation method and dried in a biological safety cabinet for 2 h at room temperature and then stored at 4 °C for 24 h to facilitate bacterial attachment before tomatoes were treated with the combined treatments of five dose levels of e-beam (0.45, 0.60, 0.75, 1.0, and 1.25 kGy) and H_2O_2 (60 mg/l) aqueous solution. The results showed that the combined treatments of 1.0 kGy and 60 mg/l achieved approximately 4-log reductions of *Salmonella* spp. The $D_{10\text{-average}}$ value of *Salmonella* spp. used in the present study was 0.25±0.01 kGy. Regarding produce quality, the combined treatments of e-beam dose up to 1.25 kGy and H_2O_2 (60 mg/l) did not affect ($P > 0.05$) the color and texture of grape tomatoes compared to nonirradiated samples. Furthermore, the combined treatments of e-beam and H_2O_2 aqueous solution reduced the dose uniformity ratio (DUR) by 6.96%.

In conclusion, exposure of the tomatoes to e-beam and water wash with added H_2O_2 has the potential to be an effective alternative decontamination system for tomato and fresh produce.

5.2 Introduction

The consumption of fresh produce has increased worldwide due to their nutritional and health-related benefits (Wadamori et al., 2016). In particular, tomatoes are the second most popular horticultural produse, next to potatoes, and are widely consumed because they are rich in health-promoting related components such as vitamins A, C, E, folate flavonoids, potassium, β-carotene, and lycopene (Martinez-Huelamo et al., 2015; Perveen et al., 2015). Due to its high antioxidant capacity, it is reported that the risk of various types of cancer and cardiovascular disease could be reduced by using the regular consumption of tomatoes (Ghavipour et al., 2015; Rowles et al., 2018).

However, the consumption of raw tomatoes has been frequently associated with foodborne outbreaks (Bennett et al., 2015; Gurtler et al., 2018). A total of 15 multistate outbreaks linked to raw tomatoes, resulting in 1959 illnesses, 384 hospitalizations, and 3 deaths, were recorded from 1990 to 2010 in the U.S. and all outbreaks were caused by *Salmonella enterica* serovars (Fan et al., 2018). In 2011, tomatoes contaminated with *S.* Newport caused 10 cases of illnesses and 3 hospitalizations in New York (CDC, 2017). In 2013, *S.* Enteritidis linked to tomatoes caused 27 cases of illnesses and 2 hospitalizations in California (CDC, 2017). In addition, the U.S. Food and Drug Administration issued 14 recalls for *Salmonella*-contaminated raw tomatoes during 2011 and 2012 (Wang and Ryser, 2014). Thus, these outbreaks have increased concerns over

postharvest decontamination practices because tomatoes can be contaminated with foodborne pathogens throughout production because of irrigation water, handling by workers, soil, and wash water (Huang et al., 2018a; Park et al., 2018).

The current decontamination practices for fresh produce such as tomatoes is predominantly based on using water with sanitizers to prevent cross-contamination and to remove soil, debris and potentially microorganism from the surface of fresh produce (Huang et al., 2018a) and to prevent cross-contamination of pathogenic microorganisms including Salmonella between contaminated and uncontaminated produce (Meireles et al., 2016). Several decontamination methods have been evaluated to inactivate foodborne pathogens in tomatoes including chlorine (Yuk et al., 2005), organic acid (Mukhopadhyay et al., 2018), electrolyzed water (Gil et al., 2015), peroxyacetic acid (Wang and Ryser, 2014; Singh et al., 2018), aerosolization of antimicrobials (Jiang et al., 2017), and aqueous and gaseous chlorine dioxide (Sao Jose and Vanetti, 2012; Sun et al., 2017). Once contaminated, tomatoes can be difficult to be cleaned by these sanitizing agents because they are very effective against pathogens including *Salmonella* on the smooth surface of tomatoes (Gurtler et al., 2018) but they do not completely inactivate Salmonella spp. located in stem scars, puncture wounds, or pulp (Prado-Silva et al., 2015). The formation of biofilm on tomato cuticles can also lead to persistence and resistance to these disinfection treatments (Kumar et al., 2018). Additionally, foodborne pathogens may be internalized in tomatoes, beyond the reach of surface sanitizers (Mukhopadhyay et al., 2018). Therefore, alternative microbial reductions strategies including irradiation need to be explored.

Ionizing radiation, such as X-rays, gamma rays, and electron beam, is a promising alternative or addition to existing methods to ensure the safety and quality of fresh produce because it has the ability to eliminate foodborne pathogens internalized in produce relative to chemical sanitizers (Gomes et al., 2009; Palekar et al., 2015). The irradiation process also has the ability to extend the shelf-life of fresh produce and reduce quality losses in fresh produce (Meireles et al., 2016; Pinela and Ferreira, 2017). Schmidt et al. (2006) found that the population of *S.* Montevideo and S. Agona on fresh-cut tomato cubes irradiated with e-beam at 0.95 kGy reduced by 2.2 and 2.4 log CFU/g. Additionally, Prakash et al. (2007) indicated that a 5 log CFU/g reduction in *Salmonella* spp. in diced tomatoes irradiated with e-beam would require a dose of 1.3-1.95 kGy because $D_{10\text{-value}}$ varied from 0.26 to 0.39 kGy. Recently, Mahmoud (2010) obtained greater than 5.0-log reduction of *S. enterica* on the surface of whole Roma tomatoes treated with X-ray irradiation at 1.5 kGy. More recently, Guerreiro et al. (2016) reported that gamma irradiation at 3.2 kGy did result in a major impact on the benefit of reducing microbiota by 2 log CFU/g after 14 days of storage at 4 °C and a potential decrease of 11 log CFU/g unit of *S. enterica* on cherry tomatoes with a significantly effect on the color of fruits. These results showed that the required treatment dose for a 5-log pathogen population reduction as recommended by the U.S. Food and Drug Administration (FDA) and the International Commission on Microbiological Specifications for Food (ICMSF) by irradiation treatment is quite high and may result in adverse effects on sensory properties and nutritional quality of tomatoes (Mahmoud, 2010; Mukhopadhyay et al., 2013). Hence, it is recommended to use irradiation treatment in combination with other

methods to reduce the irradiation doses whereas maintaining adequate antimicrobial effectiveness (Doona et al., 2015; Tawema et al., 2016)

The combination of different technologies, known as hurdle technology, has become a potential technology that may reduce losses of nutritional and sensory quality while achieving required levels of food safety with decreasing the intensity of each treatment (Park and Kang, 2015; Meireles et al., 2016; Ngnitcho et al., 2017). It has been demonstrated that the combination of spraying natural or synthetic active antimicrobial compounds with irradiation treatment was effective in increasing the killing effect of irradiation (Gomes et al., 2011; Takala et al., 2011; Tawema et al., 2016). Similarly, the combination of e-beam irradiation with Modified Atmosphere Packaging (MAP) reduced the radiation resistance of foodborne pathogens on fresh produce (Gomes et al., 2009; Moreira et al., 2012). Additionally, the treatment of UV-C light (0.6 kJ/m2) followed by low-dose gamma irradiation achieved more than 4.0 log and higher reduction (> 5 log) per tomato for *S. enterica* and *E. coli* O157:H7, respectively, on grape tomatoes (Mukhopadhyay et al., 2013). All these published researches focused on pathogens reduction on fresh produce and the impact on shelf-life of the produce after the washing water step. Hence, these proposed systems were not designed to prevent cross-contamination of the produce during washing water.

Several studies concluded that washing water served as a source of cross-contamination and sanitizing agents should be used to maintain the quality of the water and prevent cross-contamination of the produce despite their direct microbial benefit on the produce (Gil et al., 2009; Banach et al., 2015; Murray et al., 2017). In addition, when

a new hurdle technology is proposed, several parameters such as process time, water usage, the number of unit processes, and energy consumption should be considered for practical application of technology for industry (Goodburn and Wallace, 2013).

The combination of ionizing radiation and chemical treatments such as hydrogen peroxide (H_2O_2) for washing fresh produce can be a very promising tool to reduce microbial risk and prevent cross-contamination of the produce since ionizing radiation has been shown to be an effective method to remove organic pollutants and inactivate microorganisms in drinking water and wastewater treatments among advanced oxidation processes (AOPs) available such as Fenton process, TiO_2, photochemistry, and sonolysis (Taghipour, 2004; Wang and Chu, 2016; Wojnarovits and Takacs, 2017; Hong et al., 2019). H_2O_2 has been used as an alternative to chlorine for wash water disinfection in fresh produce industry because it does not produce toxic fumes in the worker space. It does not form carcinogenic disinfection byproducts and is an environmentally friendly (van Haute et al., 2015; Guo et al., 2017; Jiang et al., 2017). Additionally, H_2O_2 has been widely used in AOPs for water and wastewater treatments (Bhuiyan et al., 2016; Rozas et al., 2016; Miklos et al., 2018) because it can be converted into high oxidative hydroxyl radical (Babuponnusami and Muthukumar, 2014; Oturan and Aaron, 2014; Guan et al., 2018), which is the primary radical responsible for radiation-induced cell lethality as proved in Section 3.4.3. Therefore, to further enhance electron beam irradiation treatment for fresh produce decontamination, this physical method could be used in combination with chemical sanitizers such as hydrogen peroxide.

To the best of our knowledge, there is no available information regarding the combined use of e-beam irradiation with H_2O_2 for fresh produce decontamination. The aim of this study was to evaluate the efficacy of the combination of electron beam (e-beam) irradiation with hydrogen peroxide (H_2O_2) to inactivate Salmonella spp. in grape tomatoes.

5.3 Materials and methods

5.3.1 Fresh produces samples

Whole, fresh and unblemished (from the same lot) grape tomatoes (*Solanum lycopersicum*) were purchased at a local market (College Station, TX) the day prior to the experiment and stored at 4 °C without any washing or any sanitizing.

5.3.2 Bacterial culture

A cocktail of five *Salmonella enterica* strains was used in this study. *Salmonella enterica* subsp. *enterica* serovars (Rissen strain SAL1449, Montevideo strain SAL4599, Saintpaul strain 476398, Typhimurium ATCC strain 13311, and Typhimurium ATCC strain 700720) were provided from Dr. Castillo`s Food Microbiology Laboratory (Department of Animal Science, Texas A&M University). Frozen stocks for each *S. enterica* serovars were maintained at -80°C until further use. Prior to use, an inoculum was removed from frozen culture with a loop, streaked onto 9 mL Trypticase Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD) and incubated at 37°C for 24 h. Then, single colonies of *S. enterica* serovars isolates were obtained by streaking on Trypticase Soy Agar (TSA; Difco, Becton Dickinson, Sparks, MD) and incubated at 36 °C for 24 h

through two successive transfers on TSA. Colonies of five *S. enterica* serovars were separately stored on a TSA slant at 5 °C as working cultures and used within 30 days.

5.3.3 Inoculum preparation

In general, the inoculation procedure of Danyluk et al. (2005) and Keskinen et al. (2009) were followed with minor modifications. The inoculum was prepared by transferring a loopful of the working stock to 9 mL TSB and incubated at 37°C for 18-24 h. The overnight culture (1 ml) were spread over TSA plates (100 by 15 mm) to produce a bacterial lawn after incubation for 24±2 h at 37 °C. Four plates were prepared for each strain. After incubation, cells lawns were harvested using 10 ml of sterilized 0.1% peptone water (PW) and gently suspending the cells with a sterile plate spreader were collected in 15 ml sterile conical centrifuge tubes. The suspension in tubes were centrifuged and washed for three consecutive times (3000 x g for 15 min) with sterile deionized (DI) water at 5°C and the resulting pellets obtained for each strain were resuspended in50 mL sterilized DI water. At this point, a bacterial cocktail of all the 5 isolates of *S.* enterica serovars was prepared by mixing 50 ml each of the resuspended pellets in a sterilized beaker prior to inoculating. The average final concentration of *Salmonella spp.* in the cocktail was about 10^{10} CFU/ml as checked by plate counting on TSA. Before each experiment, fresh cultures were prepared.

5.3.4 Preparation of aqueous hydrogen peroxide solutions

Hydrogen peroxide solution (H_2O_2, 50 wt. %, Honeywell Fluka™, Honeywell International Inc., Muskegon, MI, USA) were used as received without purification. Prior to each experiment, hydrogen peroxide solutions were prepared by dissolving 60.0

mg/l into sterile 1.0 mM phosphate buffer (PB) solution. Analysis of H_2O_2 in solution was measured using drop wise titration with a standard Hach hydrogen peroxide test kit (Hach Company, Loveland, CO, USA) based on directions in the manual. The pH value of hydrogen peroxide solution was measured as 7.2±0.15 using a digital pH meter (FE20/EL20, Mettler Toledo™, Columbus, OH, USA).

5.3.5 Sample preparation and inoculation

Grape tomatoes free of visible wounds and bruises was chosen and warmed up to room temperature before bacterial inoculation. The height and length of the chosen tomatoes were 13±2.0 mm and 20±3.0 mm, respectively, and the average weight of them was 5±1.0 g.

Several studies showed that *Salmonella* spot-inoculated on fresh produce was easier to be removed by washing than dip-inoculated ones because dip-inoculated *Salmonella* has more surface area to attach and may hide in areas that could not be directly exposed to water such as tomato stem scar (Lang et al., 2004; Das et al., 2006; Sapers and Jones, 2006; Huang and Chen, 2018). Therefore, the dip inoculation method was used in the present study.

For dip-inoculation, 100 g of tomatoes were dipped in 250 ml of *Salmonella* cocktail (~10 log CFU/ml) with stirring for 1 h. Inoculated samples were then dried in a biological safety hood for 2 h at room temperature and stored at 4 °C for 24 h to facilitate bacterial attachment (Lang et al., 2004; Huang et al., 2018b).

On the day of experiment, non-sterile cylindrical sample vials (30 mm diameter) x 74 mm height) (Dynalon Labware, Dynalab Corp., Rochester, NY, USA) were initially

sterilized using 70 % ethanol and air dried in a biological safety cabinet (Class II Type A2, Labconco Corporation, Kansas City, MO, USA) for two hours (Jadhav et al., 2013; Lim and Harrison, 2016).

On the day of e-beam irradiation, three tomatoes (~15 g) inoculated with *Salmonella* spp. were placed in the sterilized sample vial for e-beam treatment. Then, approximately ~30 ml of hydrogen peroxide aqueous solution was added into each sample vial. To check the sterility of the cylindrical sample vials, three negative control samples were prepared from sterilized deionized water without bacterial suspension, before each experiment. Sample vials were sealed with Parafilm (Bemis NA, Neenah, WI, USA) and placed in heat-sealed sterile Whirl-Pak® bags (Whirl-Pak, NASCO, Fort Atkinson, WI). Because of the university biosafety regulations, these heat-sealed bags were placed inside a "specimen transport" bags that were rated up to 95 kPa (Thermosafe, Arlington Heights, IL). Afterward, samples were transported to the e-beam facility in an insulated cooler with refrigerant packs.

5.3.6 Electron beam (e-beam) irradiation treatment

The irradiation tests were carried out with a commercial scale electron beam facility managed and operated by the National Center for Electron Beam Research at Texas A&M University which houses a 10.0 MeV, 18 kW, linear accelerator. Samples were placed in a single layer in cardboard boxes (Figure 1). The actual dose absorbed by the samples was measured using alanine ($_{L}$-α-alanine pellet) dosimeters (Harwell Dosimeters, Oxfordshire, UK) and a Bruker E-scan spectrometer (Bruker, Billerica, MA, USA)

Figure 5.1 Prepared samples (A) were secured on a cardboard container (B) to be treated with the 10 MeV electron beam source. Alanine dosimeters were secured to the top and bottom of the packages. Each package contained eighteen tomatoes.

5.3.6.1 Dose mapping

The absorbed dose was measured for tomatoes irradiated at a target dose of 1.0 kGy by placing alanine dosimeters at different depths within the tomato (top (0 mm), middle (~6.5 mm), and bottom (~13 mm)). The dose uniformity ratio (DUR) is described as the ratio of maximum to minimum absorbed dose (Eq. 5.1). This value should be close to the value of 1.0 to get uniform dose distribution in samples (Moreira et al., 2012). However, values greater than 1.0 are common in commercial applications.

$$DUR = \frac{D_{max}}{D_{min}} \quad [5.1]$$

5.3.7 Bacterial enumeration

The number of surviving *Salmonella* spp. on each non-irradiated (control) and irradiated samples was enumerated. Samples not inoculated and exposed to the e-beam treatment were used to check the presence of naturally contaminated *Salmonella*. Each tomato in the vial was removed by sterile tweezers and put into a sterile sample bag. In each sample bag, 20 ml of neutralizing broth (NB) was added and then the bags were pummeled by hand until sample reduced small pieces. Samples of 1.0 ml from the original bag and 0.1 ml from serial dilution in 0.1 % of PW were plated in duplicate on TSA for 5 h with the Xylose lysine deoxycholate (XLD; Difco, Becton Dickinson, Sparks, MD) overlay for *Salmonella* spp. The plates were incubated at 37°C for 24 h (Mukhopadhyay et al., 2013). After incubation, visible colonies were enumerated with the use of a magnifier counter (detection limit was 5 CFU/g) and expressed as log CFU/g.

5.3.8 Effect of designed hurdle system on selected quality attributes of tomatoes

Samples noninoculated with *Salmonella* spp. were treated with e-beam dose of 0.45, 0.60, 0.75, 1.0, 1.25 kGy as described in Section 3.5. After irradiation treatment, the samples were stored at 10 °C for up to 6 hours. The color and texture of the tomatoes were then measured using standard methods (Prakash et al., 2002; Yun et al., 2015; Park et al., 2018).

Tomatoes were cut into small pieces and 15 g were placed in a sample cup to obtain L (lightness), a (redness to greenness), b (yellowness to blueness) readings. The color of the tomato samples (control and irradiated samples) was measured by using a LAB Scan XE colorimeter (HunterLab, Inc, VA, USA) with the HunterLab Universal software (version 3.73). The illuminant geometry was D65/10. The colorimeter was calibrated using standard white and black plates. Three readings were taken at random positions from each sample. The Hue angle (*h*) and Chroma or saturation index (*C*) were calculated as:

$$h = tan^{-1}(\frac{b}{a}) \quad [5.2]$$

$$C = \sqrt{(a)^2 + (b)^2} \quad [5.3]$$

Texture was evaluated using a TA-CT3 Brookfield Texture Analyzer (Brookfield AMETEK, MA, USA). A stainless steel probe with the diameter of 4 mm (TA 44) was used to penetrate fruit to a depth of 10 mm at a speed of 10 mm/s and a 10 kg load cell. The maximum force was recorded using the TexturePro CT software (version 1.2).

5.3.9 Microbial inactivation kinetics

5.3.9.1 Primary model

The GlnaFiT inactivation model-fitting tool (Geeraerd et al., 2005) was used to develop the microbial survival curves (CFU/ml vs. dose) for *S.* Typhimurium and calculate the inactivation model parameters for this pathogenic strain. The shoulder plus log-linear model (LLS) (Geeraerd et al., 2000) was used in this study due to its great capability to predict microbial death kinetics as proved in Section 3.4.1. The inactivation resistance parameters were calculated using the equation below

$$N_D = N_o e^{-k*SI}\left(\frac{e^{-k*SI}}{1+(e^{-k*SI}-1)e^{-k*D}}\right) \quad [5.5]$$

Where, SI is the shoulder length (kGy).

For comparison purposes, the parameter '*5D*', defined as the dose required to inactivate 99.999% of the microbial population, was used in the process design criteria as recommended for irradiation of fresh produce by Kim and Moorman (2017). The average D_{10} value, $D_{10\text{-}average}$, was calculated using the relationship below (van Asselt and Zwietering, 2006):

$$D_{10-average} = [SI + (5 * D_{10})]/5 \quad [5.6]$$

The coefficient of determination (R^2) and root mean square error (RMSE) were used to determine the goodness of fit of the primary model.

5.3.10 Experimental design

Thirty-six tomatoes were separated into two groups. The first group of eighteen were contaminated with *Salmonella* spp. as described in Section 5.3.5 to use for determining the microbial inactivation kinetic. The second group of eighteen were used for testing selected quality attributes as described Section 5.3.8. For each group, three

tomatoes in H_2O_2 aqueous solution but not treated with e-beam were used as control samples.

Three tomatoes were prepared for each point of target dose (0.45, 0.60, 0.75, 1.0, 1.25 kGy). Three replications were done for each experiment.

5.3.11 Statistical analysis

Statistical analysis was performed using the SPSS (version 20.0 for windows, 2011). The differences in quality parameters due to dose were analyzed using an analysis of variance (ANOVA) using Tukey`s multiple range tests. Statistical significance was determined at the $P<0.05$ levels.

5.4 Results and discussion

5.4.1 Dose mapping

Table 5.1 presents the dose distribution within a single tomato and in the aqueous solution. Dosimeters were placed at the middle of the tomato (6.5 mm) absorbed the highest ($P > 0.05$) e-beam dose compared to those placed at the front (0.0 mm) and back (13 mm). This result is primary due to the scattering of electrons that generates the additional absorption of dose in addition to the primary incident electrons from the e-beam. The dose uniformity ratio (DUR) was 1.15 and 1.08 for the tomato alone and that in the aqueous solution, respectively. The addition of H_2O_2 aqueous solution reduced the DUR value by 6.96%. This result is possible because the density of tomato (1.01 g/cm^3) is very close to the density of water due to its high-water content (92.3%) (Sweat, 1974). This result suggests that the combined treatment of e-beam irradiation and H_2O_2 aqueous solution would reduce the cost of processing.

Table 5.1 Dose distribution on grape tomatoes in aqueous hydrogen peroxide (H_2O_2, 60 mg/l) irradiated at 1.0 kGy as target dose with a 10 MeV electron beam source.

Single Tomato		**Tomatoes in H_2O_2 aqueous solution**	
Penetration Depth (mm)	Absorbed dose (kGy)	Penetration Depth (mm)	Absorbed dose (kGy)
0	1.28[#] (0.03)*	0	1.33 (0.02)
6.5	1.4 (0.05)	6.5	1.4 (0.04)
13	1.22 (0.06)	13	1.38 (0.02)

[#]Value are means of three dosimeters (n = 3)
*Standard deviation

5.4.2 Inactivation of Salmonella spp. on whole grape tomatoes by the combination of electron beam irradiation with hydrogen peroxide (H_2O_2) aqueous solution

The population of *Salmonella* spp. in whole grape tomatoes irradiated with e-beams was reduced ($P < 0.05$) by approximately 2.65±0.17 log CFU/g at 0.63 kGy. Schmidt et al. (2006) found similar result for the reduction of *S.* Montevideo on stem scar area of tomatoes irradiated with e-beams at 0.7 kGy. However, the authors achieved a 1.8-log reduction for *S.* Montevideo on the tomato cubes obtained from diced Roma tomatoes irradiated with e-beams at 0.7 kGy. The difference with our result is probably because the available nutrients from cut tomatoes flesh could react with hydroxyl radicals formed during radiolysis of water and increase the radiation resistance of *Salmonella* spp. present in the cubes. For example, amino acids and thiols were reported as radioprotectors (Singh and Singh, 1982). Likewise, Niemira and Solomon (2005) reported hydroxyl radicals and oxygen could be neutralizing before doing damage to bacterial cell membranes, protein structures, and nucleic acid strands, thereby protecting the bacteria in suspension with a high antioxidant capacity and reducing the efficacy of the irradiation treatment.

The population of *Salmonella* spp. on dip-inoculated whole grape tomatoes irradiated with e-beams at 0.75 kGy was reduced by approximately 3.81 log CFU/tomato. Accordingly, Mahmoud (2010) achieved 3.7 log CFU/tomato reduction of *S. enterica* on spot-inoculated whole Roma tomatoes treated with X-ray at 0.75 kGy. Similarly, Mukhopadhyay et al. (2013) obtained approximately 3.9 log CFU/tomato reduction of *S. enterica* on spot-inoculated grape tomatoes treated with gamma irradiation at 0.75 kGy.

Based on these results, in the present study, H_2O_2 did not affect the reduction of Salmonella spp. on grape tomatoes during irradiation because it may be degraded completely by enzymatic activity including catalase activity including catalase and superoxide dismutase (Kim and Thayer, 1995) before irradiation treatment due to the time (3-4 h) needed to transport samples from laboratory, where samples were prepared, to the e-beam facility and start to irradiate samples. Therefore, a faster inoculation-irradiation time must be developed.

Sapers and Jones (2006) found that the treatment of 5% H_2O_2 aqueous solution at 60 °C for 2 min reduced *Salmonella* population on tomatoes by 2.59 log CFU/g. Likewise, Guo et al. (2017) reported that a sanitizing solution of 1% H_2O_2 at room temperature for 2 min reduced the concentration of *S. enterica* on spot- and dip-inoculated grape tomatoes by 2.54 and 2.41 log CFU/g, respectively. However, in the present study, the concentration of *Salmonella* spp. on dip-inoculated grape tomatoes was not affected ($P > 0.05$) by the treatment of 0.005% H_2O_2. This difference with our result can be due to the use of low concentration of H_2O_2 (Raffellini et al., 2008) and a 24-h inoculum drying time (Sapers and Jones, 2006). Lang et al. (2004) reported that drying time affected ($P < 0.05$) survival and/or recovery of foodborne pathogens inoculated onto surface of tomatoes treated with chlorine.

The measured and predicted survival curves of *Salmonella* spp. in whole grape tomatoes in aqueous solution including 60 mg/l H_2O_2 treated with e-beam irradiation are given in Figure 5.2. The curves obtained for the treatment yielded a high correlation coefficient ($R^2 = 0.98$) and low root mean square error (RMSE = 0.30), indicating

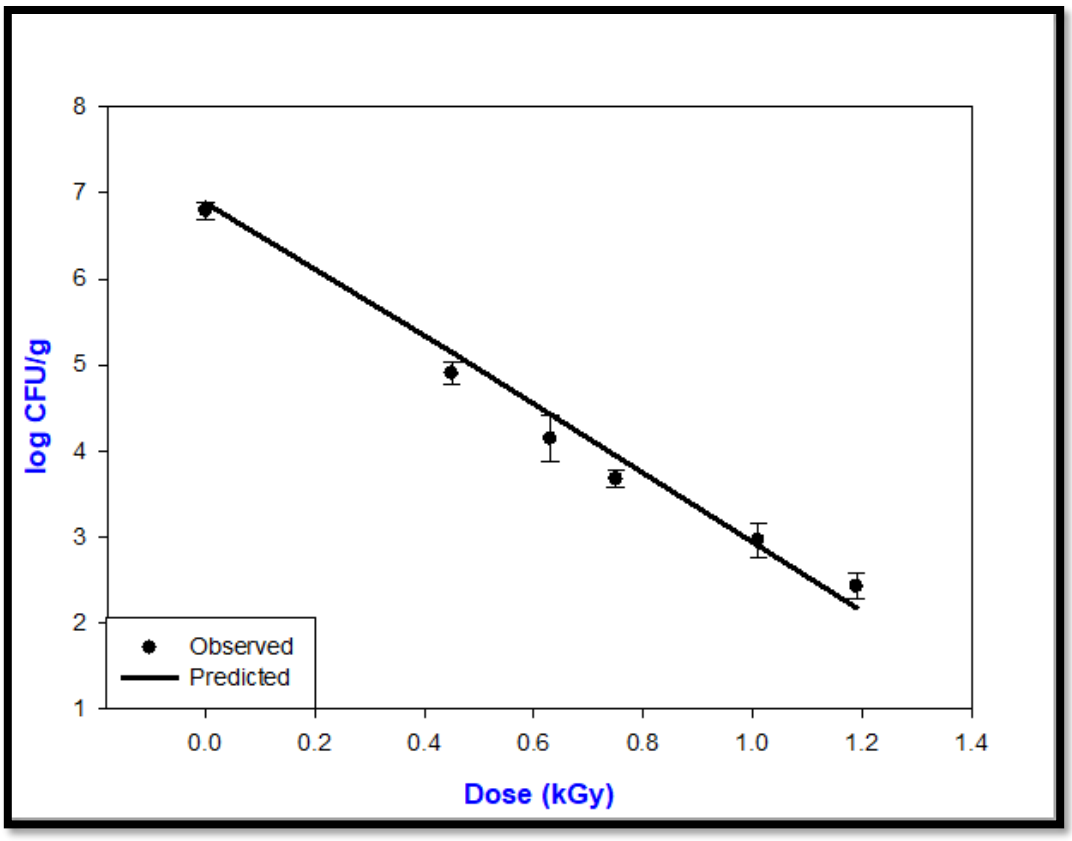

Figure 5.2 Survival curves for *Salmonella* spp. on grape tomatoes treated with the integrated treatment combining hydrogen peroxide (H_2O_2) aqueous solution and electron beam irradiation. Continuous lines represent predicted values from the LLS (log-linear plus shoulder) model (Eq. (5.6))

goodness of fit of the LLS model. The estimated shoulder length (*SI*) and inactivation rate constant (k_{max}) values were 0.021±0.03 kGy and 9.27±0.61 kGy^{-1}, respectively. Overall, the calculated $D_{10\text{-average}}$ for *Salmonella* spp. on whole grape tomatoes was 0.25±0.01 kGy (Table 5.2).

There are no published studies on the inactivation of foodborne pathogens including Salmonella in fresh produce threated with the integrated treatment combining H_2O_2 and e-beam irradiation. Table 5.2 provides the radiation resistance ($D_{10\text{-value}}$) of *Salmonella* spp. inoculated on whole or diced tomatoes and compares the current finding with reported radiation resistance data for S*almonella* when treated with ionizing radiation alone and combination with UV-C or 1% calcium chloride ($CaCI_2$). In general, these studies with the exception of Moreira et al. (2012) and Mukhopadhyay et al. (2013) have found higher D_{10} values than that obtained in the present study. One possible explanation is that DUR values could be higher than that reported in the present study. For instance, the DUR and D_{10} values obtained Guerreiro et al. (2016) for *S*. Typhimurium ATCC 14028 on cherry tomatoes irradiated with gamma ray at room temperature were 1.6 and 0.30 kGy, respectively. In addition, it is common knowledge that the radiation sensitivities differences among *S*. enterica serovars were correlated to their inherent diversity with respect to the chemical and physical structure as well as their capacity to recover from radiation injuries (Sherry et al., 2004). Prakash et al. (2007) reported that the radiation resistance of *S.* Hartford, *S*. Montevideo, and cocktail of *Salmonella* (Hartford, Montevideo, Poona, Michigan, and Gaminara) inoculated in diced tomatoes

Table 5.2 Summary of reported radiation resistance (D_{10}) of inoculated *Salmonella* on tomato and comparison with the integrated system combining hydrogen peroxide (60 mg/l) aqueous solution and electron beam (e-beam) irradiation in the present study.

Microorganism	**Types of tomatoes**	**Irradiation source**	**Dose range**	**D_{10} value**	**Source**
S. Montevideo	Chopped	E-beam	0.70-0.95	0.39	Schmidt et al. (2006)
S. Agona	Chopped	E-beam	0.70-0.95	0.54	Schmidt et al. (2006)
S. Hartforg	Diced and rinsed 1% $CaCl_2$	E-beam	0.30-0.90	0.39	Prakash et al. (2007)
S. Montevideo	Diced and rinsed 1% $CaCl_2$	E-beam	0.30-0.90	0.26	Prakash et al. (2007)
Cocktail (*S.* Hartford, *S.* Montevideo, *S.* Poona, *S.* Michigan, *S.* Gaminara	Diced and rinsed 1% $CaCl_2$	E-beam	0.30-0.90	0.32	Prakash et al. (2007)
Cocktail (*S.* Javiana, *S.* Montevideo, and *S.* Typhimurium)	Roma	X-ray	0.10-1.50	0.56	Mahmoud (2010)
S. Typhimurium LT2	Sliced	E-beam	0.20-0.90	0.17	Moreira et al. (2012)
Cocktail (*S.* Montevideo, *S.* Newport, and *S.* Stanley)	Grape	UV-C followed by Gamma	0.10-0.75	0.22	Mukhopadhyay et al. (2013)
S. Typhimurium	Cherry	Gamma	0.40-3.0	0.3	Guerreiro et al. (2016)
Cocktail (*S.* Rissen, *S.* Montevideo, *S.* Saintpaul, two strains of *S.* Typhimurium)	Grape	E-beam	0.45-1.20	0.25	In the present study

dipped in 1% calcium chloride ($CaCl_2$) irradiated with e-beams were 0.39, 0.26, and 0.32 kGy, respectively.

In addition, compared to the D_{10} value reported by Mahmoud (2010), the radiation sensitivity of *Salmonella* spp. on grape tomatoes in H_2O_2 aqueous solution irradiated with e-beams obtained in the present study increased by 55%. This result was probably due to use of different irradiation source, treatment conditions, dose rate, and the maturity and cultivar of the produce (Fan, 2012). In addition, the isolate types and strains of pathogens affect the radiation sensitivity of them (Sherry et al., 2004). Anellis et al. (1973) found that *S.* Javiana in chilled buffer was the most resistant to gamma irradiation compared to *S.* Typhimurium, *S.* Infantis, *S.* Panama, *S.* Heidelberg, and *S.* Senftenberg in chilled buffer.

Moreira et al. (2012) reported that the average DUR and D_{10} values for *S.* Typhimurium LT2 on sliced Roma tomatoes were 1.1 and 0.17 kGy, respectively. Although the DUR value was very close to that obtained in the present study, the D_{10} value was much lower than that found in the present study. These difference in D_{10} values between two studies can be due to the radiation sensitivity of each strain of *Salmonella* and the use selective media for recovery of bacteria (Sherry et al., 2004). Rodriguez at al. (2006) demonstrated that the D_{10} value of *S.* Typhimurium LT2 (0.12 kGy) was significantly less than those for *S.* Poona (0.38 kGy) when inoculated in a gelatin-based model food irradiated with e-beams.

Mukhopadhyay et al. (2013) found that the radiation resistance of *S. enterica* on grape tomatoes surface treated with UV-C followed by gamma irradiation varied from

0.19 to 0.24 kGy with a mean of 0.22 kGy, which is slightly lower than those reported in the present study. This result can be explained by the differences in inactivation mechanism of UV-C and H_2O_2. UV-C light inactivate microorganisms by generating cyclobutene pyrimidine dimers preventing DNA replication at cellular level, but H_2O_2 leads to the formation of highly reactive hydroxyl radicals attacking DNA, membrane lipids, and other essential cell components (Teksoy et al., 2011; Sun et al., 2016). Therefore, it is possible that Salmonella cells exposed to UV-C light were more vulnerable to irradiation treatment than those exposed to H_2O_2. However, Mukhopadhyay et al. (2013) did not consider preventing cross-contamination in wash water used for tomatoes and thus, the high concentration of chemical sanitizers should be still used in wash water.

In summary, the combined e-beam treatment with H_2O_2 slightly decreased the radiation resistance of *Salmonella* spp. on grape tomatoes compared to previous results (Prakash et al., 2007; Guerreiro et al., 2016). Nevertheless, it is proved that the presence of H_2O_2 increased ($P < 0.05$) the radiation sensitivity of *S.* Typhimurium ATCC 13311 in aqueous solution to e-beam irradiation in Section 4.4.2 even though the concentration of H_2O_2 decreased to below detection limit before irradiation treatment. This result may be due to fact that *Salmonella* cells adapted to oxidative stress conditions prior to irradiation treatment since H_2O_2 aqueous solution was added into samples 3-4 h before irradiation treatment. Christman et al. (1985) reported that *S.* Typhimurium cells become resistant to killing by hydrogen peroxide and other oxidants including hydroxyl radicals when pretreated with nonlethal levels of H_2O_2 because 30 proteins are induced in the 60

min following the addition of H_2O_2. As proved in Section 3.4.3, the radiation-induced cell lethality of *S*. Typhimurium ATCC 13311 in aqueous solution treated with e-beam irradiation was mainly because of hydroxyl radicals formed during radiolysis of water. Therefore, the application of the combined e-beam treatment with H_2O_2 should be applied at same time to prevent the adaptation of cells to oxidative stress.

5.4.3 The effect of combined of electron beam irradiation with hydrogen peroxide (H_2O_2) aqueous solution on quality of grape tomatoes

The combined treatment of e-beam irradiation with H_2O_2 aqueous solution did not affect ($P > 0.05$) the color parameters (*L*, *a*, and *b*) of grape tomatoes when compared to nonirradiated samples (Table 5.3). The tomatoes were red in color because the color a and hue values ranged around 14.0 and 28, respectively. These findings in agreement with the study on gamma treatment of cherry tomatoes by Guerreiro et al. (2016) who found that the color parameters of cherry tomatoes did not influenced by gamma irradiation at 1.3 kGy compared to that reported for untreated samples because color is strongly affected by fruit ripeness. In addition, compared with the nonirradiated controls, chroma values of samples irradiated at all doses were not ($P > 0.05$) different. This result suggests that there were no changes in color of tomatoes due to the applied irradiation doses. Camelo and Gomez (2004) stated that chrome was more related to consumer acceptance than hue. Regarding to obtained results, the consumer acceptance could not be affected by the irradiation treatment.

The firmness of grape tomatoes irradiated at all doses was similar ($P > 0.05$) to nonirradiated (control) samples (Table 5.3). The mean value of peak force (N) for

Table 5.3 The effect of the integrated treatment combining hydrogen peroxide (60 mg/l) aqueous solution and electron beam treatment at different dose level.

	Color parameters			**Hue**	**Chroma**	**Firmness**
Dose (kGy)	*L*	*a*	*b*	*h*	*C*	Force (Newton)
0.00	[#]16.61 *(0.54)[a]	14.36 (0.61)[a]	7.61 (0.39)[a]	27.92 (0.01)[a]	16.25 (0.68)[a]	12.25 (1.59)[a]
0.45	16.76 (0.75)[a]	14.18 (0.86)[a]	7.80 (0.56)[a]	28.82 (0.03)[a]	16.19 (0.91)[a]	12.09 (1.01)[a]
0.63	16.32 (0.75)[a]	14.18 (0.85)[a]	7.47 (0.52)[a]	27.77 (0.02)[a]	16.03 (0.94)[a]	10.49 (1.01)[a]
0.75	16.67 (0.68)[a]	14.56 (0.62)[a]	7.68 (0.48)[a]	27.80 (0.03)[a]	16.47 (0.62)[a]	10.84 (2.46)[a]
1.01	16.42 (0.81)[a]	13.93 (0.60)[a]	7.50 (0.49)[a]	28.29 (0.03)[a]	15.83 (0.57)[a]	10.46 (0.91)[a]
1.19	16.52 (0.54)[a]	14.34 (0.99)[a]	7.70 (0.39)[a]	28.31 (0.03)[a]	16.28 (0.91)[a]	10.37 (1.14)[a]

[#]Value are means of three replications (n = 9)

*Standard deviation

a: For each parameter (columns) the values between treatments have the same letters are not considered significantly (P > 0.05).

puncturing the fruits of irradiated samples was lower ($P > 0.05$) than that of non-irradiated samples, meaning that the tissue structure of the produce still remained intact although the softening of tomato fruit in response to irradiation usually occur due to some biochemical process (Ahmed et al., 1972; Akter and Khan, 2012). Similar results were reported by Guerreiro et al. (2016) who reported the firmness of cherry tomatoes did not affect by gamma irradiation up to 3.2 kGy. Nevertheless, Prakash et al. (2002) found that the firmness of diced Roma tomatoes decreased ($P < 0.05$) with increasing radiation dose (0-3.70 kGy). This difference can be attributed to the physiological condition and maturity of fruits at time of dicing (Thomas and Diehl, 1988).

5.5 Conclusion

In this study, the potential of an integrated treatment combining hydrogen peroxide (H_2O_2) aqueous solution and electron beam irradiation for decontamination of inoculated *Salmonella* spp. on whole grape tomatoes has been investigated. The results indicate that the integrated treatment improved the dose uniformity ratio (DUR) into tomatoes reducing it by 6.96%. Thus, the integrated treatment would reduce the cost of process.

The results obtained the present study show that the combined treatments of 1.0 kGy and 60 mg/l H_2O_2 achieved approximately 4-log reductions of *Salmonella* spp. In addition, that H_2O_2 applied at same time with e-beam treatment may increase the efficacy of e-beam treatment to inactivate *Salmonella* pp. in grape tomatoes due to bacterial adaptation to oxidative stress provoked by H_2O_2 (Christman et al., 1985) prior to e-beam treatment but needs further studies. Furthermore, an irradiation dose of 1.19

kGy in combination with 60 mg/l H_2O_2 did not affect ($P > 0.05$) the quality attributes of tomatoes.

Finally, the application of the combined treatments of e-beam and H_2O_2 could be an alternative decontamination system to enhance produce safety and maintain the quality characteristics of produce. Findings of this study suggest that the integrated treatment combining H_2O_2 (60 mg/l) aqueous solution with 1.25 kGy e-beam irradiation could achieve 5-log reduction of *Salmonella* without any negatively effects on the fruit quality attributes whereas current FDA regulations prohibit the application of radiation doses in excess of 1.0 kGy to fresh produce. In order to reduced required e-beam dose below to 1.0 kGy for 5 or higher log reduction, there should be an appropriate H_2O_2 additive amount coupling with irradiation procedure. Hence, further studies on the optimization of this integrated treatment are recommended.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER STUDY

This research focused on a strategy to increase the efficacy of electron beam (e-beam) irradiation to inactivate *Salmonella* spp. in whole grape tomatoes. Therefore, a hurdle decontamination process using the combination of electron beam (e-beam) and hydrogen peroxide (H_2O_2) aqueous solution and electron beam (e-beam) treatments was investigated.

The first part of this research evaluated the effect of peptone water (PW), phosphate buffered saline (PBS), phosphate buffer (PB), and the hydroxyl radical scavengers including membrane-permeable ethanol and membrane-impermeable polyethylene glycol (PEG) on the efficacy of e-beam treatment to inactivate *S.* Typhimurium ATCC strain 13311 in deionized (DI) water. The following conclusions were reached:

1. The death kinetics of *S.* Typhimurium ATCC strain 13311 used in this study irradiated in aqueous solutions was best described by the log-linear plus shoulder (LLS) model.
2. The radiation sensitivity of *S.* Typhimurium in DI water decreased ($P < 0.05$) by 19.73 % and 26.53 when PW and PBS, respectively, were dissolved into DI water.
3. Although the radiation sensitivity of *S.* Typhimurium did not change ($P > 0.05$) with the addition of 1.0 mM PB into DI water, the increased

concentration of PB, 10 mM and 50 mM in DI water decreased ($P < 0.05$) by 6.12% and 32.65%, respectively.

4. The addition of 150 mM NaCI into 10 mM PB decreased the radiation sensitivity of *S*. Typhimurium in 10 mM PB by 19.23%.
5. The radiation sensitivity of *S*. Typhimurium in 1.0 mM PB decreased ($P < 0.05$) by 65.51%, 162.07%, and 250.34% when the concentration of ethanol in the solution was modified as 78.9 mM, 394.5 mM, and 1578 mM, respectively.
6. The addition of 0.0125 mM PEG into 1.0 mM PB solution did not affect ($P > 0.05$) the radiation sensitivity of *S*. Typhimurium whereas the radiation sensitivity of the pathogen decreased by 29.66% and 43.45% when the concentration of PEG in 1.0 PB was modified as 0.125 mM and 1.875 mM PEG, respectively.

The second part of this research consisted on the effect of water quality parameters pH, alkalinity, nitrate, dissolved organic carbon (DOC) on the efficacy of e-beam treatment to inactivate *S*. Typhimurium ATCC strain 13311 in buffer solution due to their effects on the yield of hydroxyl radical produced from radiolysis of water. In addition, the combination of e-beam treatment with H_2O_2 to inactivate *S*. Typhimurium ATCC strain 13311 in buffer solution was evaluated. The following conclusions were reached:

7. Modifications of initial pH between 5.5 and 8.5 did not affect ($P > 0.05$) the effectiveness of e-beam treatment to inactivate *S.* Typhimurium in 10.0 mM PB.
8. Concentration levels of $CaCO_3$, representing alkalinity of water, did not affect ($P > 0.05$) the radiation sensitivity of *S.* Typhimurium in 1.0 mM PB.
9. The radiation sensitivity of *S.* Typhimurium in 1.0 mM PB increased ($P < 0.05$) by 6.21% and 11.72% with the addition of 100 and 1000 mg/l, respectively, nitrate into PB.
10. When 10mg/l and 100 mg/l of the organic substance (fulvic acid (FA)) were added into 1.0 mM PB, the radiation sensitivity of *S.* Typhimurium in 1.0 mM PB increased by 8.28% and 11.03%, respectively. However, the radiation sensitivity of the pathogen in buffer solution did not affect ($P > 0.05$) with the addition of 1000 mg/l FA
11. Regardless of H_2O_2 concentration, the radiation sensitivity of *S.* Typhimurium in 1.0 mM PB decreased ($P < 0.05$) by 29.66%.

In third part of this research, the potential of the integrated treatment combining e-beam irradiation and H_2O_2 aqueous solution for decontamination of inoculated *Salmonella* spp. in whole grape tomatoes was demonstrated. The following conclusions were reached:

12. The integrated treatment combining H_2O_2 aqueous solution and e-beam irradiation reduced DUR value by 6.96% when using the single side e-beam treatment.
13. The combined treatments of e-beam (up to dose of 1.19 kGy) with H_2O_2 did not affect ($P > 0.05$) the color and texture of tomatoes.

Recommendations for future research on alternative decontamination strategies to enhance the safety and extend shelf-life of fresh produce are to:

a. Verify the effect of the combined treatments of irradiation and H_2O_2 aqueous solution on the quality parameters of wash water
b. Verify the effect of the combined treatments of irradiation and H_2O_2 aqueous solution on the microbiological quality of fresh produce
c. Verify the effect of the combined treatment of irradiation and H_2O_2 aqueous solution on the quality parameters (physico-chemical and organoleptic) of fresh produce during shelf-life.
d. Study the effect of combined treatments of irradiation and H_2O_2 aqueous solution on the radiation sensitivity of foodborne pathogens such as *L. monocytogenes* and *E. coli* in fresh produce. Ultimately determine the optimum treatment to achieve 5-log reduction of foodborne pathogens while reducing the required irradiation dose below 1.0 kGy.
e. Study the effect of combined treatments of irradiation and H_2O_2 aqueous solution at high and low pH conditions on the radiation sensitivity of foodborne pathogens in fresh produce.

f. Study the effect of combined treatments of irradiation and other chemical oxidizers such as ozone, chlorine dioxide, and bromine in aqueous solution on the radiation sensitivity of foodborne pathogens in fresh produce.

g. Study the effect of combined treatment of irradiation and H_2O_2 aqueous solution under mild temperature conditions on the radiation sensitivity of foodborne pathogens in fresh produce.

h. Study the effect of combined treatment of irradiation and H_2O_2 aqueous solution including metal ions such as TiO_2 and Cu^{2+} on the radiation sensitivity of foodborne pathogens in fresh produce.

i. Determine the dynamic models to include all parameters, water quality parameters, fresh produce and environmental conditions which affected the inactivation or survival of foodborne pathogens.

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APPENDIX A

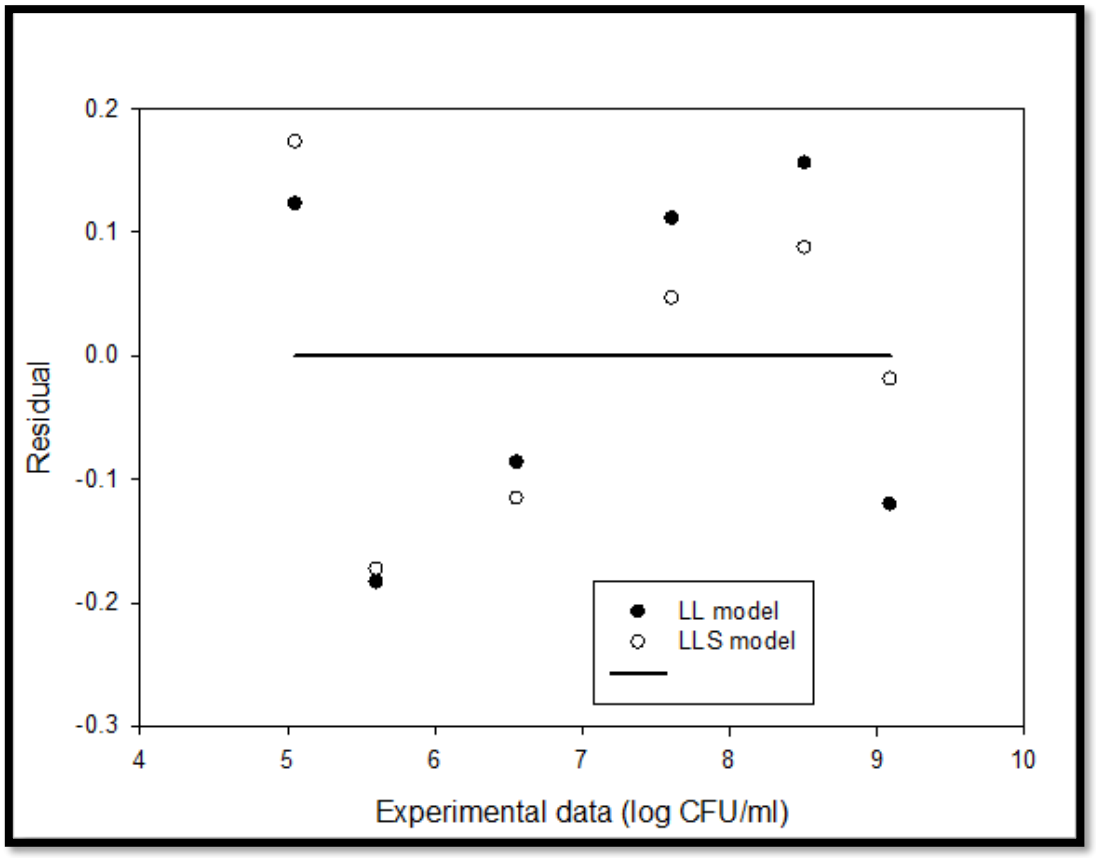

Figure A.1 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in deionized (DI) water including peptone water (PW) by electron beam irradiation at room temperature

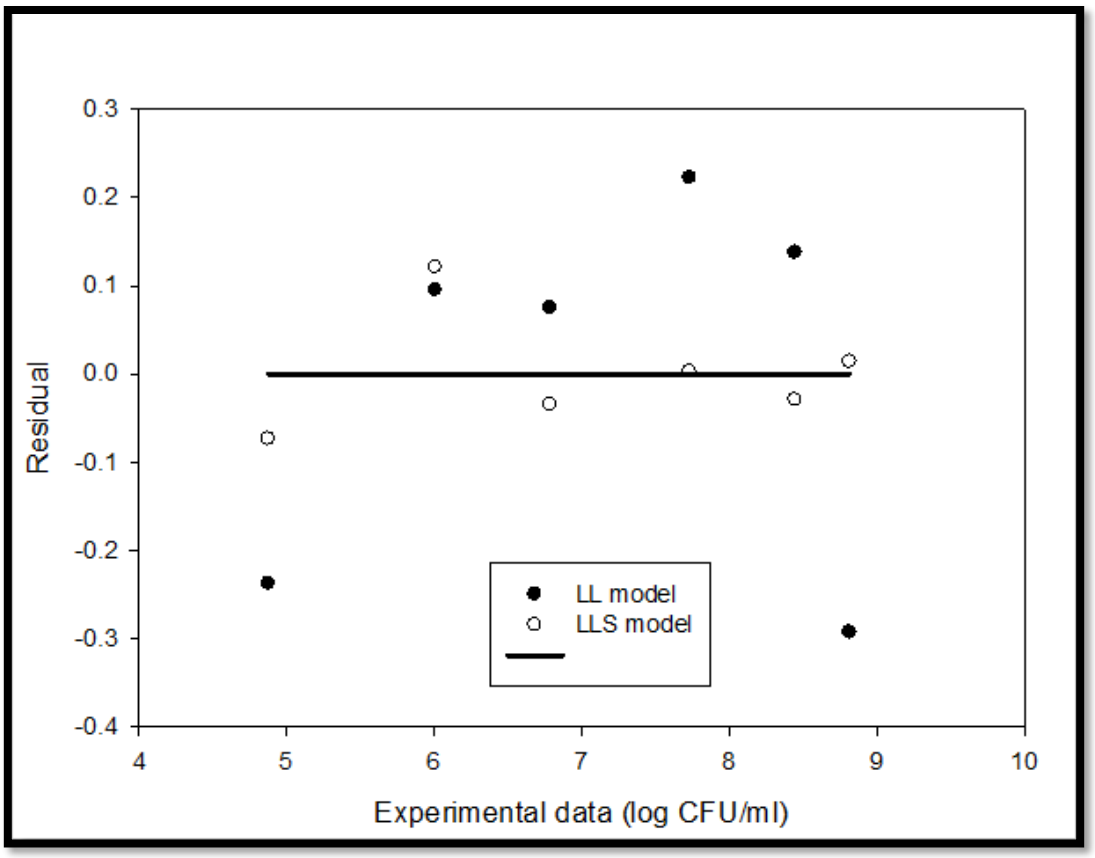

Figure A.2 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in deionized (DI) water including phosphate buffered saline (PBS) by electron beam irradiation at room temperature

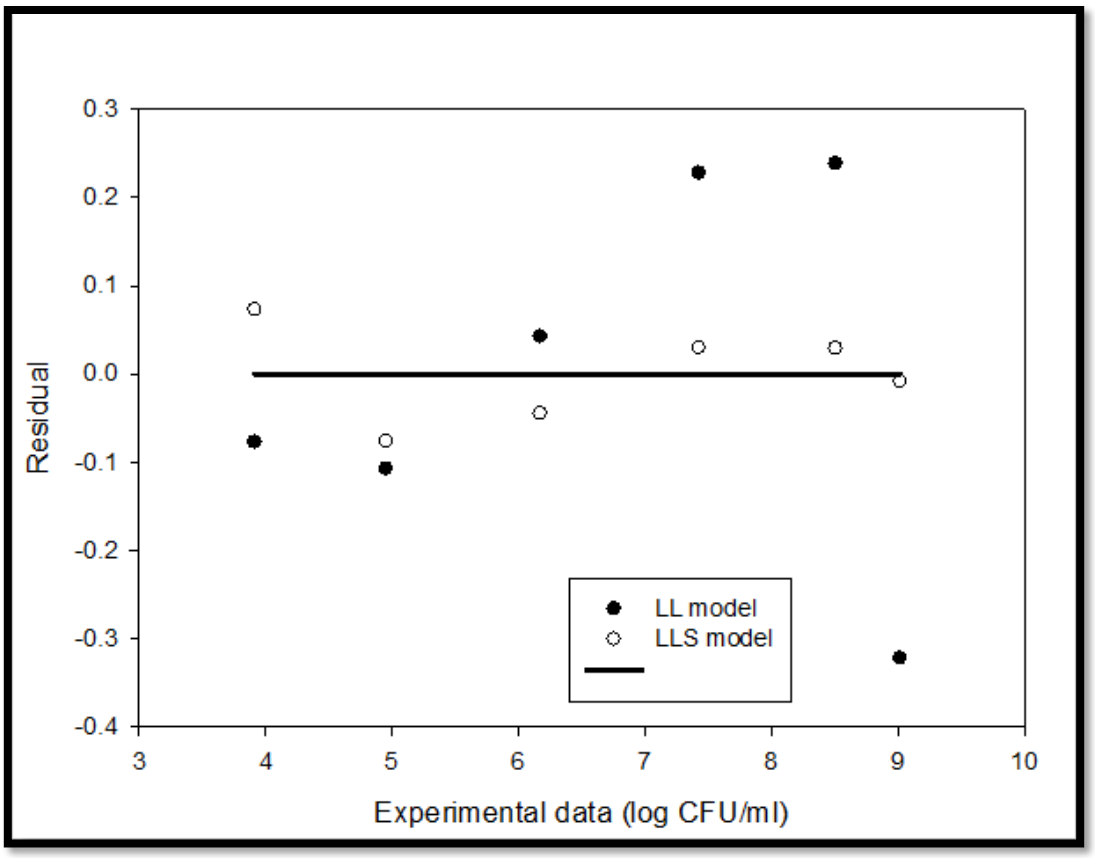

Figure A.3 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in deionized (DI) water including 1.0 mM phosphate buffer (PB) by electron beam irradiation at room temperature

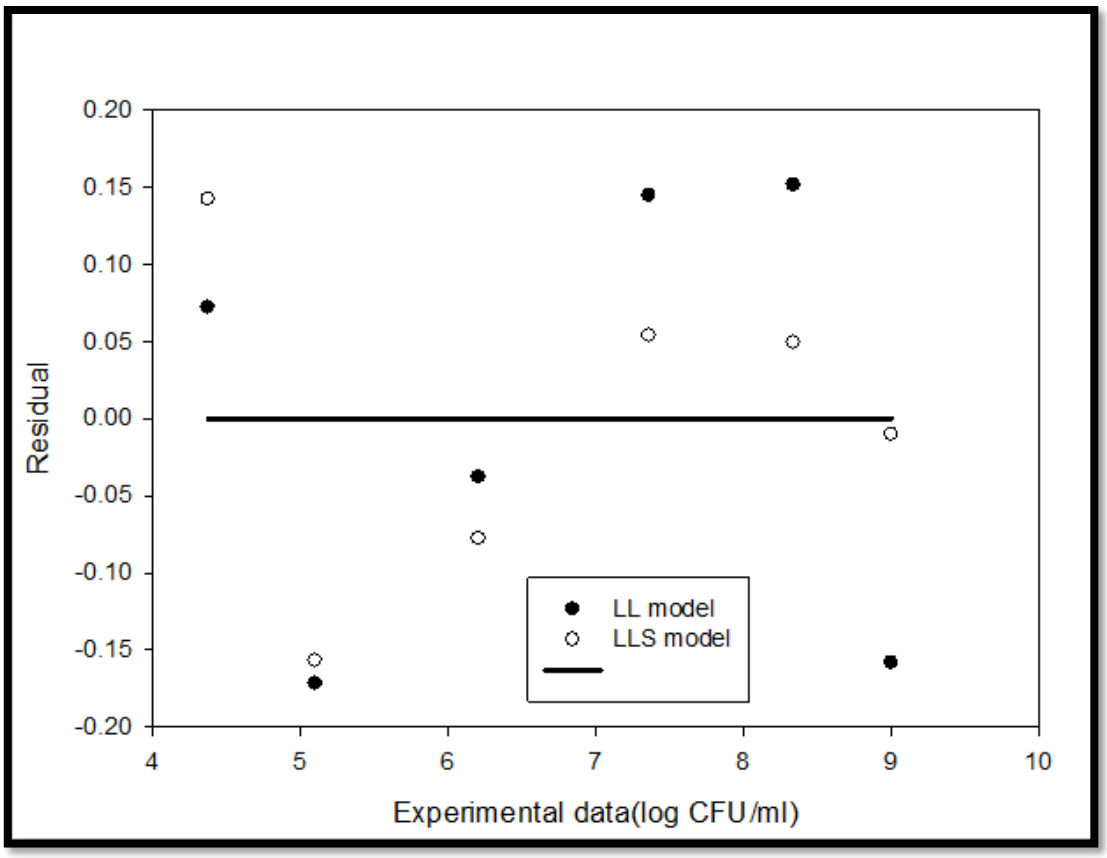

Figure A.4 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in deionized (DI) water including 10 mM phosphate buffer (PB) by electron beam irradiation at room temperature

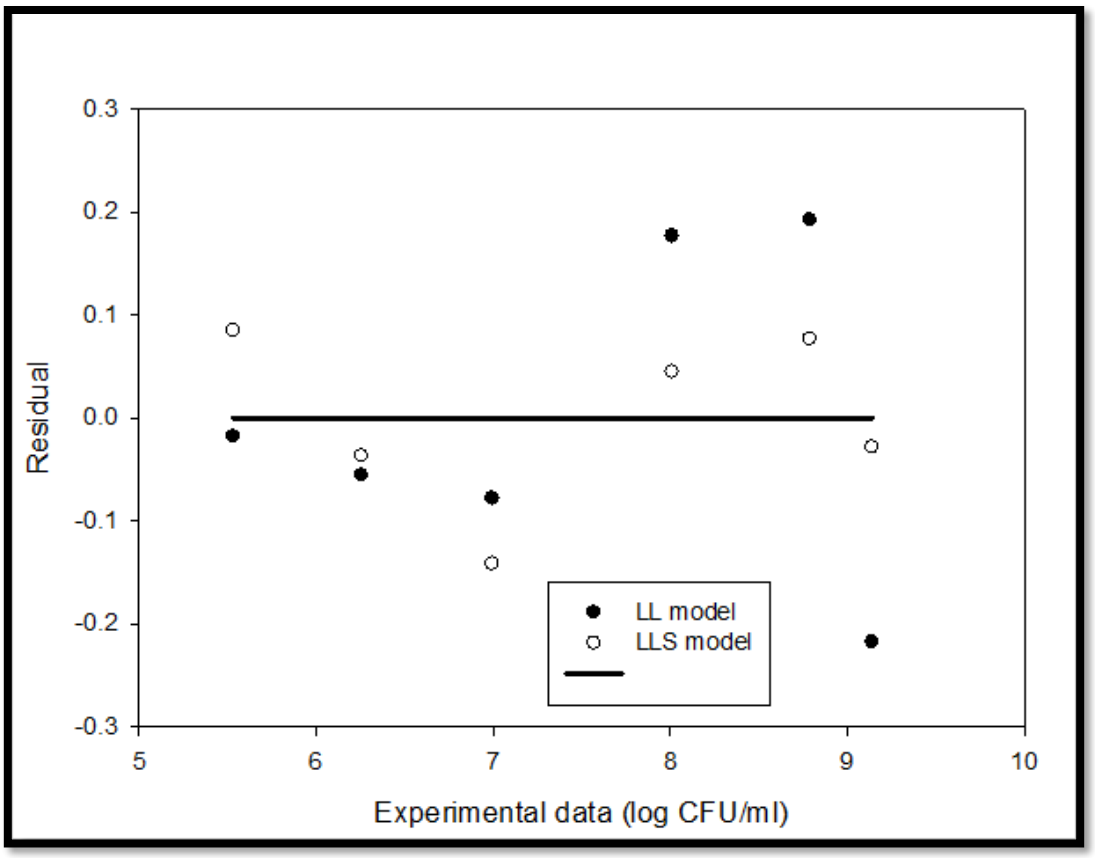

Figure A.5 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in deionized (DI) water including 50 mM phosphate buffer (PB) by electron beam irradiation at room temperature

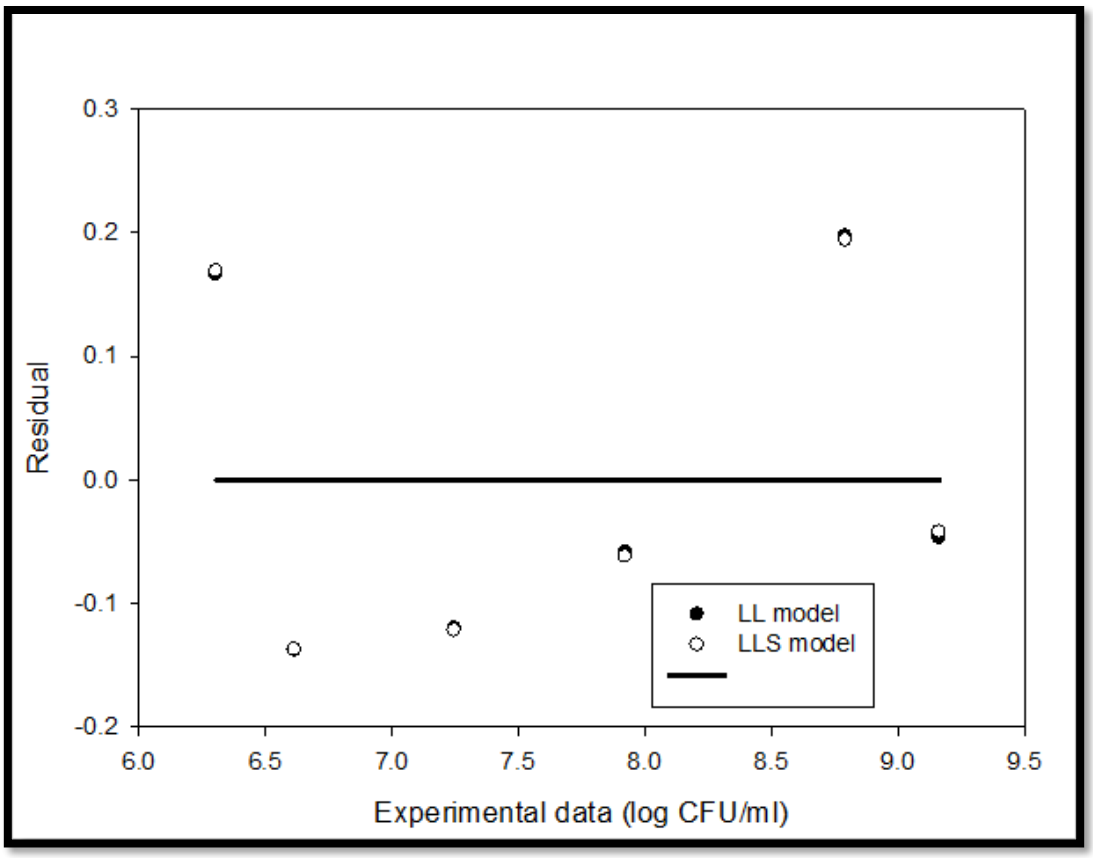

Figure A.6 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in 1.0 mm phosphate buffer (PB) including 98.9 mM ethanol by electron beam irradiation at room temperature

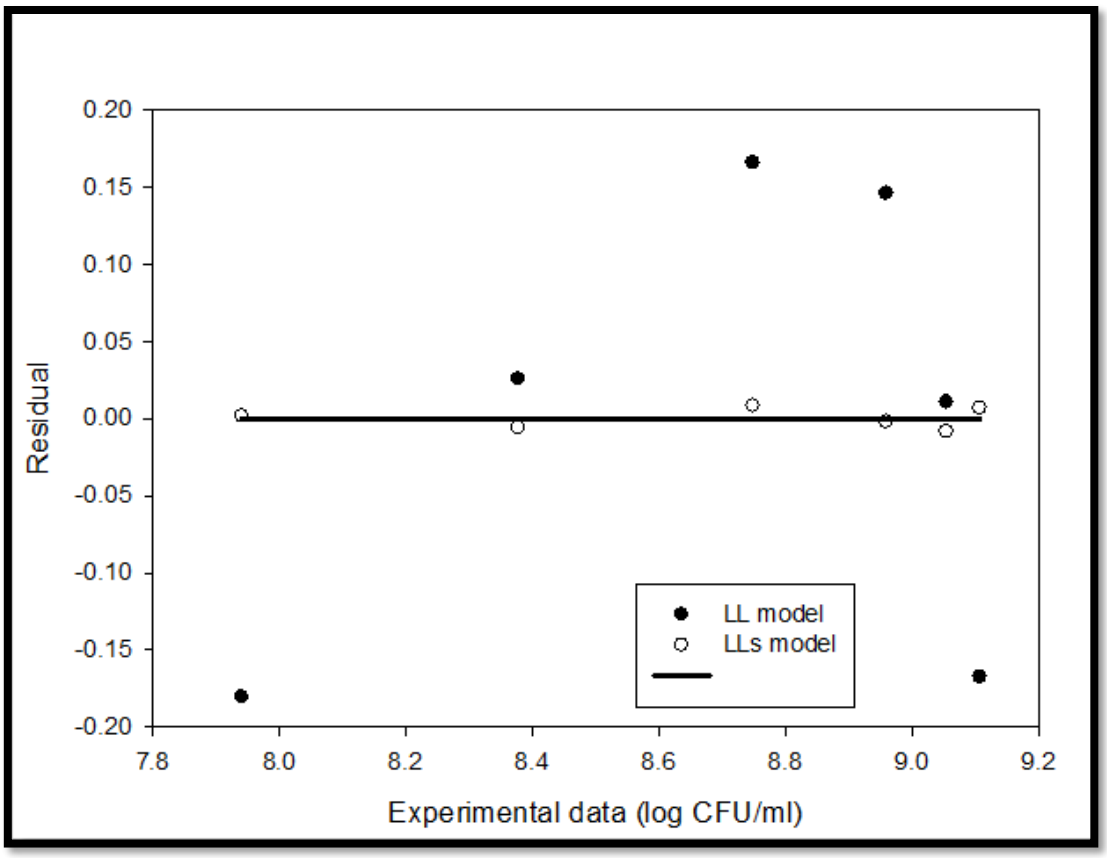

Figure A.7 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in 1.0 mM phosphate buffer (PB) including 394.5 mM ethanol by electron beam irradiation at room temperature

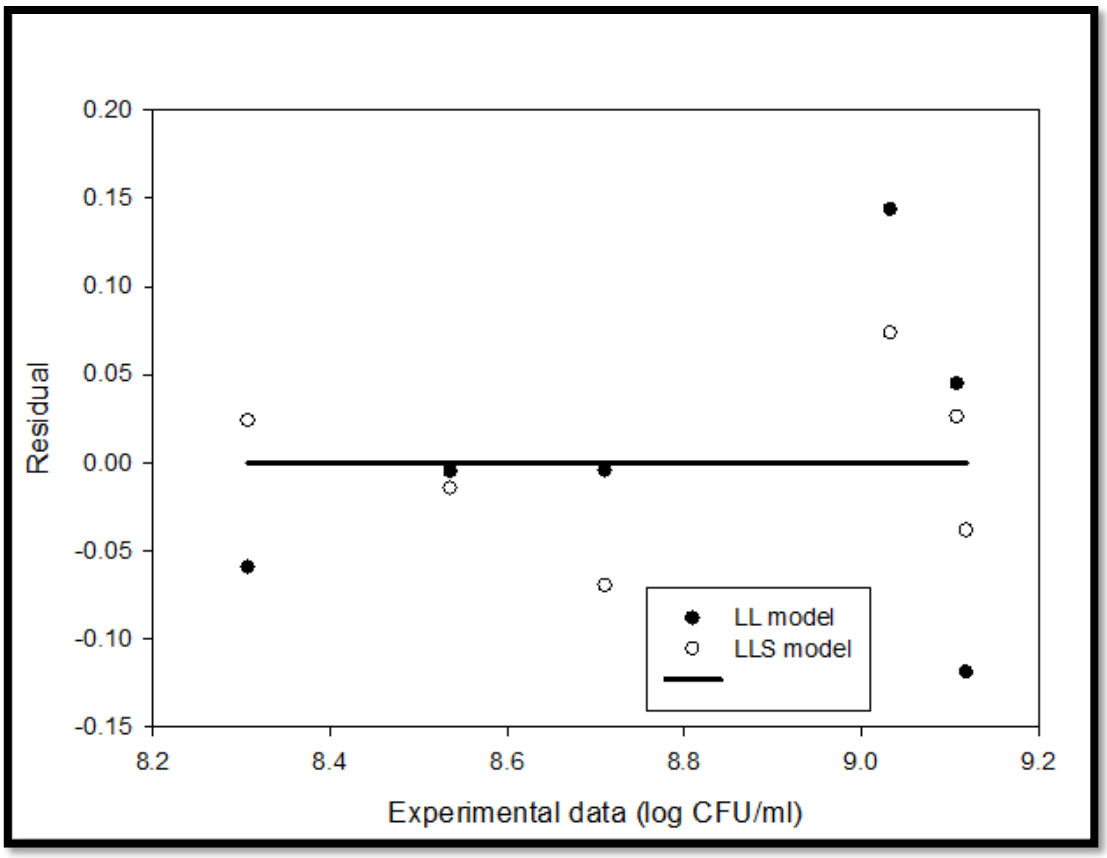

Figure A.8 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in 1.0 mM phosphate buffer (PB) including 1578 mM ethanol by electron beam irradiation at room temperature

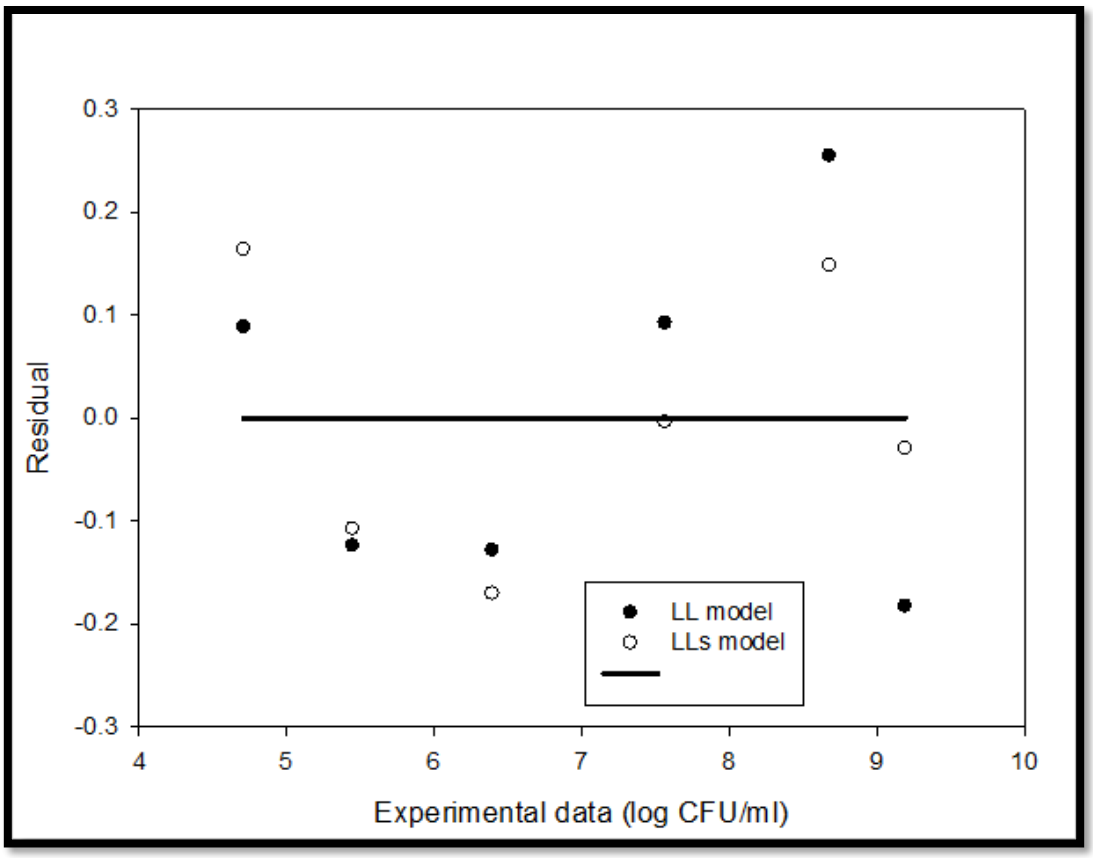

Figure A.9 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in 1.0 mM phosphate buffer (PB) including 0.0125 mM polyethylene glycol (PEG) by electron beam irradiation at room temperature

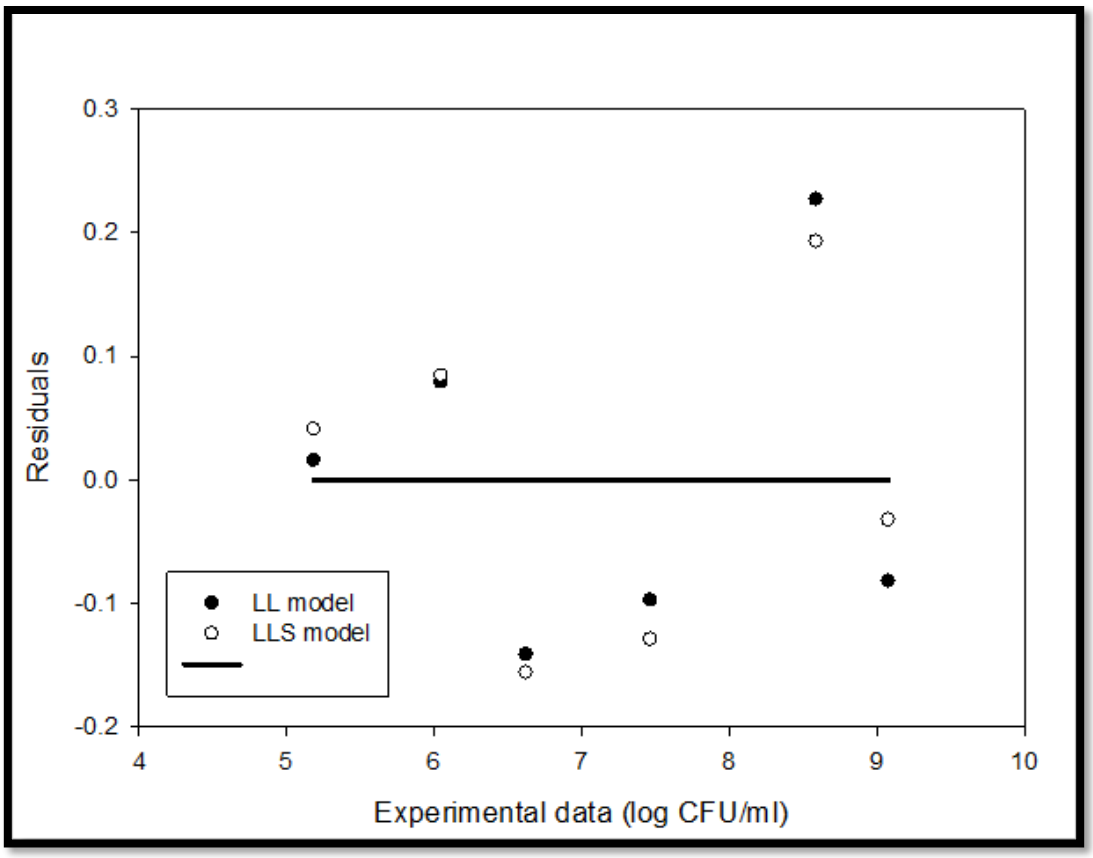

Figure A.10 Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in 1.0 phosphate buffer (PB) including 0.125 mM polyethylene glycol (PEG) by electron beam irradiation at room temperature

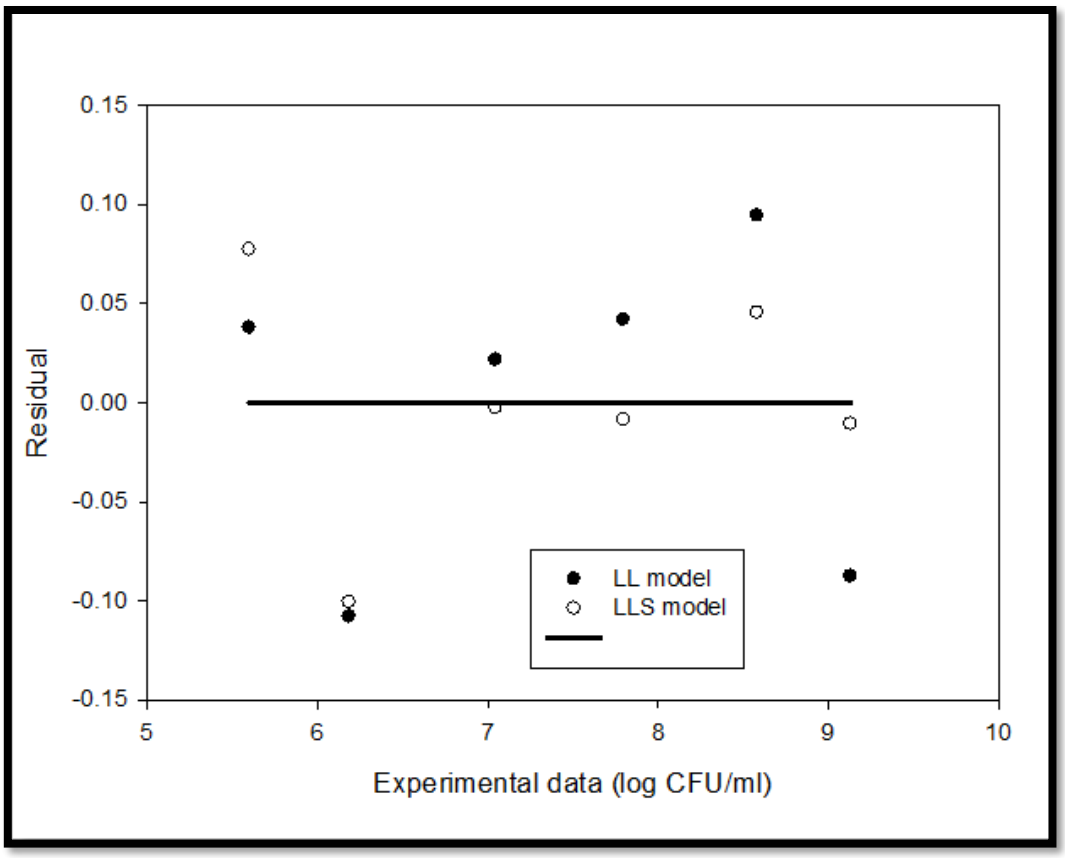

Figure A.11 . Residuals plots for the log-linear (LL) and log-linear plus shoulder (LLS) models for the inactivation of *S.* Typhimurium ATCC strain 13311 irradiated in 1.0 mM phosphate buffer (PB) including 1.875 mM polyethylene glycol (PEG) by electron beam irradiation at room temperature