EFFECT OF LOW-DOSE X-RAY AND E-BEAM IRRADIATION ON *ESCHERICHIA COLI* O157:H7, NON-O157 (VTEC) *ESCHERICHIA COLI* AND *SALMONELLA* VIABILITY ON MEAT SURFACES AND SENSORY QUALITY OF MEAT

By

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DEDICATION

To my Grandparents, Ma and Kéven.

ORGANIZATION OF THE THESIS

This thesis is divided into seven chapters and includes three manuscripts, which are included as chapter 3, 4 and 5. These chapters were standardized for presentation in thesis format.

The first chapter gives a brief introduction about the use of irradiation against *E. coli* O157:H7, non-O157 VTEC *E. coli* and *Salmonella*. General and specific objectives are described at the end of this chapter.

The second chapter presents a comprehensive literature review with a more detailed examination of the use of irradiation in food, its applications and the significance of each group of the test pathogens.

Chapter three was submitted for peer review and publication as a research note in the Journal of Food Protection. Authorship is by D. Kundu, A. Gill and R. A. Holley, and the paper is entitled "Use of Low-Dose Irradiation to Evaluate the Sensitivity of *E. coli* O157:H7, non-O157 VTEC *E. coli* and *Salmonella* in Phosphate Buffer".

Chapter four summarizes the results of the meat trials performed by inoculating the test pathogens on sliced beef and irradiated at 1 kGy. This was submitted to Meat Science, entitled "Use of low dose e-beam irradiation to reduce *E. coli* O157:H7, non-O157 (VTEC) *E. coli* and *Salmonella* viability on meat surfaces" and is by D. Kundu, A. Gill, C. Lui, N. Goswami and R. Holley.

Chapter five describes the sensory analysis performed using raw carcass muscles and cooked ground beef patties with a trained panel. This was submitted to the Journal of

Food Science, entitled “Effect of low-dose electron beam irradiation on quality of ground beef patties and raw, intact carcass muscle pieces” and is by D. Kundu and R. Holley. The study was done with two types of beef products, cooked ground beef with varying amounts of irradiated meat in the formulation and four types of raw carcass muscles.

Chapter six summarizes the main conclusions of all the studies. Finally, chapter seven presents some recommendations for future research.

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ABSTRACT

Radiation sensitivity of *E. coli* O157:H7, non-O157 VTEC and *Salmonella* to low-dose ionizing irradiation was evaluated. Buffer-suspended *E. coli* O157 and non-O157 VTEC strains showed similar resistance to 300 Gy X-ray treatments, while *Salmonella* strains were more resistant. A dose of 1 kGy E-beam radiation reduced two groups of non-O157 *E. coli* mixtures and one *E. coli* O157:H7 group inoculated in meat by at least 4 log CFU/g. *Salmonella* showed only a <2 log CFU/g reduction. Sensory attributes of cooked ground beef patties were not affected ($p > 0.05$) by irradiation. However, irradiated raw carcass muscles were more brown ($p < 0.05$) but displayed less intense off-aroma ($p < 0.05$) compared to the control during storage. Therefore, a 1 kGy treatment has the potential to improve microbiological safety with minimal effects on sensory properties of beef; it would be a suitable method for treating carcass trim before preparing ground beef.

CHAPTER 1

1. Introduction

As emerging pathogens continue to be more widespread and virulent, the demand for novel, portable, non-thermal decontamination methods is on the rise. Verotoxigenic *Escherichia coli* (VTEC) and *Salmonella* are recognized as major food-borne pathogens worldwide. As cattle are natural hosts for these pathogens, contamination during processing of the meat often causes these pathogens to be introduced as adulterants (Bosilevac & Koohmaraie, 2011; Gill & Gill, 2010; Bosilevac et al., 2009; Rhoades et al., 2009). Contaminated beef and undercooked ground beef were the most commonly reported vehicle for sporadic and outbreak illnesses (Rangel et al., 2005). Treatment of foods with ionizing radiation is considered to be a safe and an effective method for inactivation of pathogens (Niemira et. al., 2002; Black & Jaczynski 2006). Several studies have been conducted to demonstrate the effectiveness of using ionizing radiation to minimize populations of *E. coli* O157:H7 and since the organism is associated with large outbreaks, it has been extensively studied by the scientific community (Tarr, 1995). However, there are at least 200 other non-O157 VTEC *E. coli* that are potentially as pathogenic as *E. coli* O157:H7 (Fratamico & Smith, 2006). Similarly, *Salmonella* is less frequently identified as a source in outbreaks, although its prevalence in beef may often be higher than that of *E. coli* O157:H7 (Bosilevac et al., 2009; Rhoades et al., 2009). A study by Arthur et al. (2005) demonstrated a possible future for the use of a low dose (≤1 kGy) and a low-penetrating (≤ 15 mm) electron beam treatment as a method of surface decontamination of beef carcass surface cuts, but the main purpose of this

present study was to validate for the first time the potential of low dose e-beam treatment to eliminate non-O157 VTEC from cuts of trim from carcasses to be used in hamburger manufacture. To our knowledge, there is no information which characterizes the radiation resistance of non-O157 VTEC and very little information which describes the sensitivity of a diverse group of *E. coli* O157:H7 and *Salmonella* strains to allow a reliable estimate to be made of the efficacy of low dose e-beam treatment of surface-contaminated beef. Therefore, the main objectives of this study were (i) to evaluate the sensitivity of 6 serovars of *Salmonella*, 5 strains of *E. coli* O157:H7 and 27 strains of non-O157 VTEC *E. coli* (representing 19 serotypes) to a low dose 1 kGy e-beam irradiation treatment, (ii) to determine the extent to which 1 kGy e-beam radiation is able to reduce the viability of multi-organism cocktails of the more radiation resistant strains/serovars of these pathogens on the surface of fresh beef pieces and (iii) to determine whether an absorbed dose of 1 kGy e-beam radiation has detectable effects upon the sensory characteristics of beef patties containing different levels of fat and differing amounts of irradiated ground beef, or upon the appearance of four carcass muscles (outside flat, inside round, brisket and sirloin).

CHAPTER 2

2. Literature review

2.1. Irradiation

2.1.1. Food irradiation

Radiation may be defined as energy in the form of electromagnetic waves or as subatomic particles that are capable of moving through space with different wavelengths and varying degrees of power. There are two types of radiation, non-ionizing and ionizing. Light, infrared heat, and microwaves are forms of non-ionizing radiation (CFIA, 2012a). The form of radiation of interest here is ionizing radiation. Ionizing radiation may be particulate (alpha and beta particles) or in the form of waves in nature (X-rays, gamma rays). Ionizing radiation is produced by unstable atoms, which in turn give rise to charged atoms by removing electrons from their orbit.

Food irradiation is the process of exposing food products to controlled amounts irradiation. This is capable of inhibiting vital cell functioning of microorganism by destroying DNA, RNA and other macromolecules that are essential for their survival. The three different types of radiation currently allowed in the food industry are gamma rays, X-rays and electron beams (WHO, 2012; USEPA, 2012).

As the world's population is on the rise, there is greater demand to ensure the safety and to improve the shelf life of food, especially perishable foods such as meat. In the year 1995, a loss of nearly 3.5 billion kg of poultry and meat occurred in the United States alone at the consumer, retail and foodservice levels and a majority of the loss was attributed to microbial spoilage (Kantor *et al*., 1997). Reports from all over the world

suggest that a major threat in regards to microbial spoilage is food-borne illnesses caused by consumption of contaminated products. This necessitates appropriate remedial measures to ensure the safety of the public and to improve economic productivity. The use of food irradiation may be an effective solution (Käferstein, 1990). Studies on the application of ionizing radiation to food began in the early 1950s. Since then this technique has been vigorously researched by the scientific community (Olson, 1998).

2.1.2. Applications of irradiation

The need to consider the wholesomeness of irradiated food was first brought up in 1961 by the joint committee of Food and Agriculture Organization (FAO), the International Atomic Energy Authority (IAEA), and the World Health Organization (WHO) and by 1981 they concluded that a dose of up to 10 kGy was safe for human consumption with no negative side effects. The most important aspect that is considered regarding food irradiation is the dosage used. The absorbed dose should not be higher or lower than the level required to achieve the desired effect while maintaining the quality of food. There are guidelines for the minimum and the maximum dosage that can be used based on the objective. Irradiation is effective in low doses (≤ 1 kGy) to inhibit sprouting in crops, to destroy insects and parasites and to delay ripening and spoilage of fruits and vegetables. Medium doses (≤ 10 kGy) can be used for reduction of non-spore forming microorganisms that would improve shelf life of perishable products and eliminate pathogens. High dose levels between 10- 50 kGy can be used for dry herbs and spices and for commercial sterilization (WHO, 1981; Crawford & Ruff, 1996).

Irradiation at low to medium doses acts in a similar manner to conventional heat

pasteurization. It can reliably reduce and eliminate certain pathogens such as *Salmonella*, *Shigella*, *E. coli,* and *Campylobacter* (Crawford & Ruff, 1996; Käferstein, 1990). As irradiation at controlled doses does not completely eliminate all spoilage microorganisms from food, it does not replace the need for proper food handling practices.

From the nutritional aspect, irradiation at low doses causes insignificant nutrient losses, while the losses at medium and high doses maybe greater but they can be successfully minimized by excluding air or conducting the irradiation at temperatures below freezing (WHO, 1981).

Since 1986, it is mandatory to identify irradiated pre-packaged foods with the international radiation or radura symbol (Fig. 2.1).

Figure 2.1: International Radiation Symbol

Source: CFIA (2012a)

2.1.3. Major differences between X-rays and e-beam accelerators

There are two sources of ionizing radiation that were utilized in this study, X-rays and e-beam.

Electron beam (e-beam) utilizes high-energy electrons for pasteurization or sterilization instead of heat and high-pressure (Tahergorabi et al., 2012). The process involves accelerating electrons to the speed of light by a linear accelerator and transferring these high energy electrons onto a food product via an e-beam gun, resulting in microbial inactivation (Fig. 2.2). Pasteurization or sterilization can be achieved by varying the e-beam dose. E-beam processing does not alter the temperature of processed food. Therefore, e-beam processing does not cause food quality degradation due to heat. Unlike gamma radiation which uses radioisotopes, the electron source in e-beam irradiation is electricity. Hence, there is no need for hazardous radioactive isotopes to be present in the processing facility. The downside to e-beam processing compared to gamma radiation is its limited penetration (~4 cm). Hence, the dimensions and size of the food products that are to be irradiated have to be carefully considered. The irradiation dose is generally measured in Grays (G) or kiloGrays (kGy), where 1 Gray = 0.001 kGy = 1 Joule (J) of energy absorbed per kilogram (kg) of irradiated food.

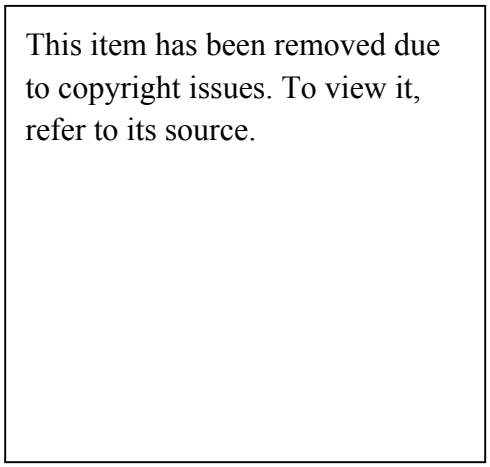

Figure 2.2: E-beam generated by a linear accelerator

Source: Tahergorabi et al. (2012)

X-rays are generated when a metal target is interposed between the e-beam and processed food (Fig. 2.3). The high-energy electrons in the e-beam from the linear accelerator impinge upon the metal target and produce X-rays. In the case of X-rays the radiation source is similar to an e-beam, and is also a linear accelerator. At an energy level of 5 MeV, the efficiency of converting an e-beam into X-rays is about 10% of the incident e-beam power. The advantage of using X-rays is that they have penetration capability similar to gamma rays (Tahergorabi et al., 2012).

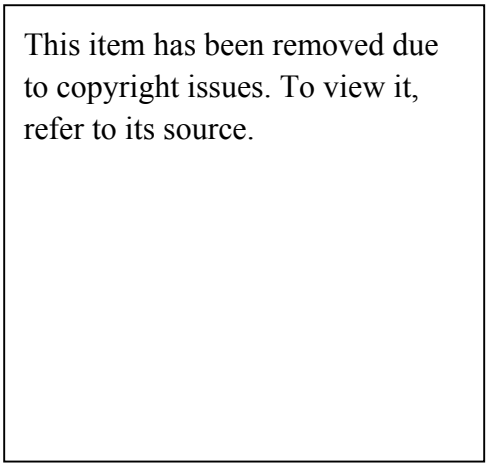

Figure 2.3: X-rays generated by an e-beam striking a metal target

Source: Tahergorabi et al. (2012)

2.2. Verotoxigenic *E. coli*

Verotoxigenic *E. coli* (VTEC) are Gram-negative bacilli that cause illness by producing a toxin referred to as a verotoxin or shiga-like toxin, hence they are also known as shiga toxin-producing (STEC) *E. coli*. These organisms have the ability to produce one or two shiga toxins, stx_1 or stx_2, and attach to intestinal mucosal cells (Karmali, 1989). They are very resistant to environmental stresses and can withstand cold storage, acid conditions and drying (AHW, 2011). The illness caused by VTEC occurs in two phases; the first phase is characterized by abdominal pain, diarrhea, nausea, emesis and fever. The next phase is the elaboration of the toxin which often leads to complications such as hemorrhagic colitis and haemolytic uremic syndrome (HUS) which are characterized by acquired non-immune haemolytic anemia, thrombocytopenia, and acute renal failure. Illness most commonly affects children, the elderly and the immune-

compromised. The significance of this illness lies in its severity and not in numbers of cases, as the organism has an infective dose of 10 cells and the overall case fatality rate is about 1%. (Tarr, 1995; AHW, 2011).

2.2.1. Significance of *E. coli* O157:H7 and non-O157 VTEC *E. coli*

Beef cattle are considered to be reservoirs of both *E. coli* O157:H7 and related non-O157 VTEC *E. coli* (Hussein, 2007). The importance of beef safety increased after an outbreak in 1982 in the United States when patients were diagnosed with hemorrhagic colitis after eating ground beef contaminated with *E. coli* O157:H7 from a fast food chain restaurant (Riley et al., 1983). Since then *E. coli* O157:H7 has been extensively studied and identified as the most prevalent VTEC serotypes in North America and Europe (Tarr, 1995). From 1982 to 2002, 52% of the 350 outbreaks reported caused by these organisms were food-borne and of these 41% were linked to the consumption of ground beef and other beef products (Rangel et al., 2005). In Canada, from 2000 to 2004, 6505 cases of VTEC illness were reported to the National Enteric Surveillance Program (NESP), of which 94% were caused by serotype O157 (PHAC, 2009). Recently, the largest beef recall in Canada occurred, involving nearly 1 million kg of fresh beef contaminated by *E. coli* O157:H7. (Huffington Post, 2012). In the province of Manitoba alone there were 267 reported cases of VTEC illness from 2004 to 2006 (MBH, 2007). Normally the identification of *E. coli* O157 involves utilizing its typical phenotypic features such as its lack of sorbitol fermentation. However, there are several other non-O157 VTEC *E. coli* that have the same pathogenic features but several of these non-O157 strains ferment sorbitol. A simple biochemical test that allows non-O157 VTEC *E. coli* to be easily distinguished and identifiable is lacking, and this often leads to misdiagnosis

(Bielaszewska & Karch, 2000). A study in Manitoba concluded that 63% of the VTEC infections were caused by non-O157 which were mostly sporadic cases (Thompson et al., 2005).

Overall there has been an increasing awareness about the importance of VTEC other than the O157 serotypes and the total number of reported cases of non-O157 STEC infections increased from 171 in 2000 to 501 in 2005 in the United States, suggesting a higher burden of non-O157 VTEC than initially thought. From 1990 to 2006 there were 13 outbreaks caused by non-O157 VTEC *E. coli* in the United States. In fact 20-50% of all VTEC cases may be caused by non-O157 VTEC *E. coli* (Johnson et al., 2006). In 1994, *E. coli* O157:H7 was declared to be an adulterant in ground beef and routine sampling and testing was required of the beef industry (USDA, 2007). In 2012 6 non-O157 VTEC serotypes, O26, O103, O45, O111, O121 and O145 were identified as major threats and the FSIS declared that these 6 serotypes would be added to the routine testing of ground beef (USDA, 2012).

2.3. Significance of *Salmonella*

Salmonella has also been identified as a major cause of gastroenteritis in North America. It has been estimated to cause more than 1.3 million cases of food-borne illnesses each year, accounting for 9.7% of food-related cases, and is second after *Campylobacter* (Mead et al., 1999). Similar to verotoxigenic *E. coli, Salmonella* spp. can also have a very low infectious dose of about 15-20 cells (Saulo, 2007), and cattle are a typical reservoir for these organisms (PHAC, 2011). It is of interest that consumption of ground beef was linked to a recent multi-state outbreak of *Salmonella* Typhimurium

infections in 7 different states in the USA 2011, resulting in a recall of an undetermined amount of fresh ground beef (CDC, 2012). Bosilevac et al. (2009) reported the overall prevalence of *Salmonella* in commercial ground beef samples to be 4.2% but in 94.2% of samples *Salmonella* were present at levels below 2 CFU/g. The most common strains observed were *Salmonella enterica*, serovars Montevideo, Anatum, Muenster and Mbandaka.

2.4. Challenges with ground beef

It has already been well established that *E. coli* and *Salmonella* survive in the gut of cattle, which serves as a reservoir for these organisms. Although it is necessary to follow steps to maximize the microbial safety of beef during processing, it is highly likely that contamination may occur during slaughter and carcass breaking operations, as these pathogens are naturally present in cattle (Edwards & Fung, 2006; Bosilevac & Koohmaraie, 2011; Gill & Gill, 2010; Rhoades et al., 2009).

The vulnerability of ground beef with respect to safety is mainly due to the fact that it is non-intact. In intact beef cuts, such as steaks, roasts, briskets, and stew beef, the presence of microorganisms is mainly on the surface of these cuts as long as the animal was healthy. Since the tissue remains intact, the possibility of pathogens migrating to the inside is very low, and the contamination of the surface can be taken care of by cooking at temperatures commonly used by most consumers. In non-intact cuts, the beef muscles undergo grinding, chopping or mincing, which transfers the microorganisms that were on the surface to the internal tissues. Meat thus contaminated must be fully cooked throughout to be safe. With processes used in the preparation of non-intact meat, chances

of cross-contamination also increases if the equipment used is not sanitized properly. If contaminated meat is not cooked to an internal temperature of 71 °C (CFIA, 2012b) pathogens may survive and cause illness when consumed (USDA, 1999b).

2.5. Effect of irradiation on quality of ground beef

Although the effectiveness of ionizing radiation is very well documented it has yet to be approved for the decontamination of fresh meat and poultry in Canada. However, in the United States, the U.S. Food Safety and Inspection Service has approved the use of ionizing radiation to a maximum dose of 3.0 kGy for poultry, 4.5 kGy for refrigerated meat and 7.0 kGy for frozen meat since 1997 (USDA, 1999; 2008). These restrictions on doses are based on the maximum dosage required to destroy specific pathogens. Clavero et al. (1994) estimated that a dose of 2.5 kGy would be sufficient to kill 10^8 CFU/g *E. coli* O157:H7 and 10^3 CFU/g *Salmonella.* Studies with gamma irradiation at 1 and 2 kGy have shown significant reductions in the number of *E. coli* O157:H7, while a dose of 3 kGy effectively eliminated the pathogen (4 log unit reduction) (Badr, 2005). Several studies found that irradiation shows promise in effectively reducing the population of a variety of pathogens. But with any new food treatment, the main question remains about consumer acceptance. Irradiation is also known to produce off-odours and off-flavours in food products including beef by the formation of radiolytic products that are dependent on radiation dose, dose rate, packaging conditions, and temperature (Thayer, 1990). In beef, pork, ham and chicken, the formation of volatile hydrocarbons have been documented which is related to the fat content of the meat, regardless of origin (WHO, 1981). Radiolytic type compounds can also form naturally in foods during cooking without irradiation (Chen et al., 2002). Fortunately, the factors that determine the amount

of radiolytic products formed can be controlled and the treatment optimized in a manner to minimize microbial load while maintaining the sensory quality of meat. Additionally, adding antioxidants and other substances, like α-tocopherol can substantially reduce the formation of off-odours (Sohn et al., 2009). Irradiation of meat at a dose of up to 1 kGy causes minimal sensory changes (Arthur et al., 2005). However, the detection of off-odours and off-flavours increased from doses ≥ 2 kGy (Thayer, 1993).

Ideally, in practice, doses as low as 1 kGy can cause a 3 to 4 log reduction of *E. coli* O157:H7 in beef (Arthur et al., 2005; Peirson et al., 2005). But the problem with ground beef or non-intact beef is that it requires high-penetration and high-energy radiation to ensure that the meat product has completely been exposed on the surface as well as the internal regions (Arthur et al., 2005). However, as contamination in a healthy animal is on the surface, applying irradiation as a method of surface decontamination of carcass trim to be later ground would allow the successful use of a low-dose and a low-penetration irradiation method to eliminate the pathogens without compromising the organoleptic and nutritional qualities of beef.

CHAPTER 3

Use of Low-Dose Irradiation to Evaluate the Sensitivity of *E. coli* O157:H7, non-O157 VTEC *E. coli* and *Salmonella* in Phosphate Buffer

3.1. Abstract

Verotoxigenic *Escherichia coli* (VTEC) and *Salmonella* are major food-borne pathogens, but there is very little information available to describe the radiation resistance of a sufficiently diverse group of these pathogens. The objective of this study was to evaluate the sensitivity of *E. coli* O157:H7, non-O157 VTEC and *Salmonella* to a low-dose ionizing irradiation treatment. Test organisms included 6 serovars of *Salmonella*, 5 strains of *E. coli* O157:H7 and 27 strains of non-O157 VTEC *E. coli* (representing 19 serotypes). Decimal reduction dose (D) values of individual strains were determined for cells in phosphate buffered saline using an X-ray source. The viability of the bacterial cells declined with an increase in absorbed dose from 0 to 300 Gy. The test organisms were screened at 500 Gy and 700 Gy for the more resistant strains. All 6 *Salmonella* strains survived at 500 Gy and 700 Gy, however only 11 VTEC survived at 700 Gy. Both O157 and non-O157 VTEC showed a similar range of D values with no significant difference ($p > 0.05$) following the 300 Gy treatments, ranging from 28-123 and 37-127 Gy, respectively. *Salmonella* strains showed a slightly higher range of D values (from 61 to 147 Gy), which was significantly higher ($p < 0.05$) than both the *E. coli* O157:H7 and non-O157 VTEC groups.

3.2. Introduction

Verotoxigenic (VTEC) *Escherichia coli* O157, non-O157 VTEC and *Salmonella* have emerged as major food-borne pathogens which consistently cause gastrointestinal illnesses in North America. Most of the outbreaks caused by VTEC have been linked to the consumption of undercooked ground beef (Boyce et al., 1995). Since *E. coli* O157:H7 is often responsible for life-threatening illnesses, it has been identified as a major target by the beef processing industry and has received considerable attention from the scientific community. Unfortunately, there are about 200 serotypes of VTEC *E. coli* that are currently known which share the same clinical, pathogenic and epidemiologic features with *E. coli* O157:H7, and have the potential to cause hemorrhagic colitis and the hemolytic-uremic syndrome (Fratamico & Smith, 2006). Twenty-three outbreaks of non-O157 VTEC illnesses were reported in the U.S. between 1990 and 2007 (Grant et al., 2011). By 2010 the number of reported illnesses caused by non-O157 VTEC reached over 1500 (USDA, 2011). In most outbreaks the non-O157 VTEC are unidentified because suitable methods for their detection and characterization are incompletely validated and are not widely available. It is difficult to separate pathogenic non-O157 VTEC from non-pathogenic *E. coli* because isolates from each group rarely have any distinguishing biochemical or phenotypic features. The U.S. Centers for Disease Control and Prevention (CDC) observed that the non-O157 *E. coli* serogroups O26, O103, O45, O111, O121 and O145 were responsible for the largest numbers of illnesses caused by non-O157 VTEC in the U.S (CDC, 2007). The USDA-FSIS announced that ground beef and precursor products would be routinely analyzed for the previous 6 non-O157 VTEC strains along with O157 VTEC in regulatory ground beef testing protocols starting in

mid-2012. The agency indicated that contaminated products would be prevented from entering commerce (USDA 2011; 2012).

The use of radiation from radioactive isotopes, electron beams or X-rays to eliminate pathogens from food has acceptance to varying degrees in different countries. Ionizing radiation is suitable for the decontamination of fresh meats as it does not substantially raise the internal temperature of foods at bactericidal dosages (Black & Jaczynski, 2006). Irradiation at up to 10 kGy has been established to pose no toxicological, microbiological or nutritional hazard by the Joint Expert Committee on Food Irradiation of the Food and Agriculture Organization, the International Atomic Energy Authority, and the World Health Organization (WHO, 1981).

Currently there is little information available that characterizes and describes the radiation sensitivity of non-O157 VTEC and *Salmonella* strains to low-dose irradiation treatment. Such information would be valuable to assess its potential for controlling VTEC contamination of beef trim by treatment before processing into ground beef. Therefore, the objective of this study was to evaluate the sensitivity of different strains of *E. coli* O157:H7, non-O157 VTEC and *Salmonella* to low-dose irradiation treatment. A further goal was to generate *in vitro* data, which would allow a more informed choice of representative strains for use in future studies on meat decontamination.

3.3. Materials and Methods

3.3.1. Bacterial strains

Six strains of *Salmonella* and 32 strains of *E. coli* were obtained from the Public Health Agency of Canada (Winnipeg, MB, Canada) and the Bureau of Microbial

Hazards, Health Canada (Ottawa, ON, Canada) for this study. All the *E. coli* strains used tested positive for one or more shiga toxin genes (stx_1, stx_2) as determined by PCR (Paton & Paton, 2003). Cultures were stored at -75 °C in glycerol-supplemented media, and streaked for growth on Brain Heart Infusion Agar (BHIA, Difco, Becton Dickinson and Co., Sparks, MD, USA), and then incubated at 37 °C for 24 to 48 h.

3.3.2. Preparation of cell suspensions

For radiation resistance determinations, individual strains of *E. coli* and *Salmonella* were suspended in buffer and treated with X-ray radiation, which was used because of its greater accuracy in low-dose delivery to buffer-suspended cells.

Turbidity measurements were used to standardize the density of cell suspensions prior to irradiation experiments. Each strain was grown to the stationary phase by independently inoculating a single colony into 25 mL of BHI Broth, which was incubated overnight at 37 °C with shaking at 100 rpm. The cells were washed by centrifugation at 11,000 × g for 10 min and re-suspended in 25 mL of phosphate buffered saline (PBS), pH 7.0. The cell suspension was standardized by dilution in PBS to an optical density between 0.095 and 0.100 at 600 nm. Two mL aliquots of the cell suspensions were transferred to sterile 2 mL capacity polypropylene cryogenic vials (Corning Inc., Corning NY, USA). The cell suspensions were maintained on ice up to 2 h for transport to the irradiation facility.

3.3.3. X-ray irradiation

An X-RAD 320 kVp, 12.5 mA unit (Precision X-Ray, Inc., North Branford CT, USA) equipped with a 2 mm aluminium filter to block non-homogenous energy

generated, located at the Health Canada Radiation Protection Bureau (Ottawa, ON, Canada) was used to screen cultures for radiation resistance. The distance from the source to the object was 32.2 cm with a field size of 13.3 x 13.3 cm. The resulting dose rate was 5.90 Gy/min; therefore, 8.5 min was required to deliver a 50 Gy dose. The experiments were carried out at ambient temperature and atmospheric pressure which ranged from 21.5 °C to 28.5 °C and 741 to 766 mm Hg.

3.3.4. Survival curves of irradiated bacteria

Each strain was held on ice before and after exposure to irradiation doses of 50, 100, 150, 200, 250, and 300 Gy. After exposure all samples, along with the untreated control, were serially diluted in PBS and plated in duplicate on selective and non-selective agar media using a spiral plater (Don Whitley Scientific Limited, West Yorkshire, UK). BHIA was used as the non-selective media, and MacConkey Agar (Difco) or Brilliant Green Agar (Difco) were used as selective media for *E. coli* and *Salmonella,* respectively. Agar plates were incubated at 37 °C for 16 to 24 h. Duplicate experiments were conducted with all strains, and triplicate experiments were used for the strains showing higher resistance at 300 Gy. The relative resistance of each strain was assessed by determining the decimal reduction dose (D), calculated as the negative reciprocal of the slope for each regression line (Clavero et al., 1994).

3.3.5. Screening for radiation resistance

In order to characterize the more radiation resistant strains, 2 mL aliquots of the cell suspension were exposed to a total dose of 500 Gy or 700 Gy. To determine survival, 1 mL of the irradiated samples and untreated controls were transferred to 10 mL of BHI

Broth and incubated for 16 to 24 h at 37 °C with shaking at 100 rpm. The tubes were checked for turbidity, and growth was confirmed by streaking on BHI Agar. The untreated controls were serially diluted and plated to determine the initial bacterial numbers.

3.3.6. Statistical Analysis

All statistical analyses were performed with JMP® 8.0.1 (SAS Institute Inc.). The decimal reduction dose (D) values between the two media were compared using Student's t tests with $\alpha = 0.05$.

3.4. Results and Discussion

Following X-ray treatment the number of viable cells suspended in buffer declined with increased irradiation dose for all strains. All but 5 of 32 *E. coli* strains and all 6 strains of *Salmonella* survived doses up to 500 Gy. At 700 Gy, 11 *E. coli* strains (Table 3.1) and all *Salmonella* strains (Table 3.2) survived. Previously reported D values for *E. coli* O157:H7 irradiated in acidified BHI Broth (pH 4.0 to 5.5) were between 50 to 120 Gy (Buchanan et al., 1999). In the present study, the D values of *E. coli* O157:H7 strains were between 28 to 123 Gy, with the highest value being for *E. coli* O157:H7 EO122. Among the non-O157 strains, the highest D value was 127 Gy for *E. coli* O145: NM 04-7099 and the lowest (37 Gy) was for *E. coli* O111:NM CFS3 (Table 3.1). Therefore, both the non-O157 and O157 VTEC groups showed a similar range of D values with no significant difference. *Salmonella* strains were used here for comparison and they showed relatively higher resistance ($p < 0.05$) than the *E. coli* strains, with D values ranging from 61 to 147 Gy (Table 3.2). The *Salmonella* strains and 7 of the 11 strains of VTEC *E. coli*

that survived exposure to 700 Gy were observed to have significantly greater numbers of colonies on the non-selective than on the selective media. This was taken to indicate that a greater proportion of cells in cultures showing higher irradiation resistance had sub-lethal injury, as opposed to lethal injury.

Previous studies have demonstrated that bacterial D values are usually higher in food samples than in buffer or broth. The sensitivity of organisms to irradiation is affected by several factors, such as water activity, level of oxygen present, and type of suspension medium (Moreira et al., 2012). For example, *E. coli* O157:H7 C9490 showed D values of 160-240 Gy in apple juice (Buchanan et al., 1998), 326-339 Gy on Boston, green or red leaf lettuce, and iceberg lettuce homogenates (Niemira et al., 2002), but only 100-120 Gy in acidified BHI broth (Buchanan et al., 1999). These results and the observation that mixtures of strains often showed higher resistance than individual strains (Sommers & Rajkowski, 2008) may mean that injury recovery is facilitated in more nutrient rich media and that beneficial interspecies interactions or selection of more resistant phenotypes occurred.

It has also been demonstrated that bacteria are more sensitive to irradiation in buffer than in a more nutrient rich medium like broth. This could be attributed to the lethal effect of hydroxyl ions produced from water adjacent to the outer bacterial cell membrane. However, if the suspension medium was broth, the organic components of the broth would be capable of minimizing the damage by rapidly scavenging the free radicals or reversing changes by donating hydrogen ions (Shenoy et al., 1975). Thus, the lethal responses observed here in buffer will likely change when these organisms are irradiated on meat surfaces. Protective effects from irradiation damage would be expected because

of the antioxidant activity of the meat as well as from any modified meat package atmosphere that might be used. However, more important than the absolute D values reported here is the observation that there was no significant difference in radiation sensitivity between the two groups of VTEC examined, each of which was more sensitive to irradiation than the *Salmonella* strains tested. On the basis of results presented, it is highly likely that low-dose radiation treatment sufficient to control *E. coli* O157:H7 will be adequate for the control of non-O157 VTEC of current concern. Study to verify this hypothesis for fresh beef is underway.

Table 3.1: Mean decimal reduction dose (D) values of VTEC *E. coli* strains exposed to 300 Gy X-ray radiation in phosphate buffered saline (PBS) and enumerated on Brain Heart Infusion Agar (BHIA) or MacConkey Agar (MAC) and culture viability after exposure to 500 and 700 Gy.

Serotype	Strain	D value (BHIA)	Std	D value (MAC)	Std	Survival following 500 Gy	700 Gy
O5:NM	03-2835	106.86 [A]	3.93	89.05 [B]	3.86	+	+
O6:H34	03-5166 [1]	75.03	2.17	75.13	2.91	+	+
O26:H11	00-3941	54.47	1.14	56.28	0.17	+	-
O26:H11	01-5870	65.50 [A]	1.21	56.28 [B]	0.17	+	-
O26:H11	02-6738 [1]	99.15 [A]	1.79	92.47 [B]	1.46	+	+
O45:H2	04-2445 [1]	87.84	2.50	85.42	2.29	+	+
O45:H2	05-6545	101.85	19.51	97.79	10.14	+	-
O55:H7	05-0376	71.82	0.57	66.30	1.80	-	-
O85:H11	03-3638	107.99	5.53	103.55	0.03	+	-
O91:H21	85-489	60.95	0.11	ND [2]	ND	+	-
O103:H2	99-2076	53.54 [A]	1.07	41.32 [B]	0.27	+	-
O103:H2	04-2446	55.53	3.94	52.51	0.09	+	-
O103:H2	01-6102	52.95	2.05	56.88	12.05	+	-
O111:NM	98-8338	86.29 [A]	1.45	56.68 [B]	3.88	-	-
O111:NM	CFS3	39.66	2.25	36.57	0.07	+	-
O111:NM	CFS4	47.14 [A]	1.10	41.20 [B]	0.58	-	-
O113:H21	93-0016	68.78	1.09	57.62	3.66	+	+
O115:H18	03-3645 [1]	84.28 [A]	8.96	64.49 [B]	4.48	+	+
O117:H7	02-4495	78.46	13.24	56.11	2.69	+	-
O121:H19	00-5288	45.04	0.73	47.31	0.98	+	-
O121:H19	03-2642	70.52	16.30	58.21	4.19	+	-
O121:H19	03-2832	70.50	1.72	60.55	4.76	+	-
O145:NM	03-4699	61.70	10.25	57.42	4.17	+	-
O145:NM	04-7099 [1]	127.06 [A]	2.12	119.25 [B]	3.41	+	+
O145:H2	75-83	80.38	2.35	78.17	7.63	+	-
O157:H7	161-84	33.43	1.02	28.69	0.15	-	-
O157:H7	CO283	54.44	5.40	48.49	0.19	+	+
O157:H7	1931 [1]	87.93 [A]	1.12	80.35 [B]	3.33	+	+
O157:H7	1934 [1]	74.61 [A]	0.43	64.59 [B]	2.51	+	+
O157:H7	EO122 [1]	123.17 [A]	1.77	107.44 [B]	1.14	+	+
O165:H25	00-4540	49.12 [A]	1.08	37.25 [B]	1.70	-	-
O177:NM	03-3974	74.62	3.45	75.07	3.37	+	-

[1] D values for strains are means of three replicate experiments and the remainder are means of duplicate experiments. Std – standard deviation. Different letters in rows indicate significant differences in the D values determined on BHIA and MAC with a Student's t-test ($P < 0.05$).

[2] Strain did not grow on the selective media.

Table 3.2: Mean decimal reduction dose (D) value[1] of *Salmonella* strains exposed to 300 Gy X-ray radiation in PBS buffer, enumerated on Brain Heart Infusion Agar (BHIA) or Brilliant Green Agar (BGA) and culture viability after exposure to 500 and 700 Gy.

Name	Strain #	Dvalue (BHI)	Std (BHI)	Dvalue (BG)	Std (BG)	Survival following 500 Gy	700 Gy
S. Enteritidis	107	95.8	1.66	ND[2]	ND	+	+
S. Muenster	184	61.1	1.73	78.9	2.06	+	+
S. Typhimurium	1193	93.9	0.98	99.7	1.84	+	+
S. Newport	1194	102.5	1.33	112.7	1.46	+	+
S. Montevideo	02-4092	147.2	4.08	137.5	2.03	+	+
S. Derby	NA	123.5	3.21	109.1	3.25	+	+

[1]D values are the mean of three replicated experiments. Std – standard deviation. D values determined on BHI and BG were significantly different for all strains as determined by Student's t-test ($P < 0.05$).

[2] Strain did not grow on the selective medium.

CHAPTER 4

Use of low dose e-beam irradiation to reduce *E. coli* O157:H7, non-O157 VTEC *E. coli* and *Salmonella* viability on meat surfaces.

4.1. Abstract

This study determined the extent that irradiation of fresh beef surfaces with an absorbed dose of 1 kGy electron (e-) beam irradiation might reduce the viability of mixtures of O157 and non-O157 verotoxigenic *Escherichia coli* (VTEC) and *Salmonella.* Groups of these pathogens were inoculated into beef surfaces (outside flat and inside round, top and bottom muscle cuts), and then e-beam irradiated. *Salmonella* serovars were most resistant to the 1 kGy treatment, showing a reduction of < 2 log CFU/g. This treatment reduced the viability of two groups of non-O157 *E. coli* mixtures by ≤ 4.5 and ≤ 4.0 log CFU/g. Reductions of ≤ 4.1 log CFU/ g were observed for *E. coli* O157:H7 cocktails. Since under normal processing conditions the levels of these pathogens on beef carcasses would be lower than the lethality caused by the treatment used, irradiation at 1 kGy would be expected to eliminate the hazard represented by VTEC *E. coli*.

4.2. Introduction

Verotoxigenic *Escherichia coli* (VTEC, also known as Shiga toxin producing or STEC) and *Salmonella* are major causes of food-borne gastrointestinal illnesses in North America. Cattle are hosts for these pathogens and a significant proportion of beef may become contaminated during slaughter and carcass breaking operations (Bosilevac & Koohmaraie, 2011; Gill & Gill, 2010; Bosilevac et al., 2009; Rhoades et al., 2009). Contaminated meat is a potential source of both sporadic and outbreak illness. Beef was the most commonly reported vehicle for foodborne outbreaks of VTEC serotype

O157:H7 illness in the USA from 1982-2002, accounting for 41% of outbreaks (Rangel et al., 2005). In addition to O157 VTEC, there are over 200 additional serotypes of non-O157 VTEC which are now recognised as potential human pathogens (EFSA, 2007; USDA, 2011; Grant et al., 2011). The source of sporadic cases of VTEC illness are rarely identified and are commonly misdiagnosed, due to the technical complexity of their isolation, but non-O157 VTEC outbreaks attributed to beef products have been reported (CDC, 2010; King et al., 2010; Ethelberg et al., 2009; Rangel et al., 2005; Werber et al., 2002). In response the USDA-FSIS announced that ground beef and precursor products would be routinely analyzed for the VTEC serogroups O26, O45, O103, O111, O121 and O145 (USDA 2011, 2012). These serogroups have previously been found responsible for 70% of reported VTEC illness in the USA (Brooks et al., 2005). As with non-O157 VTEC, the prevalence of *Salmonella* in beef is often reported to be higher than that of *E. coli* O157 (Bosilevac et al., 2009; Rhoades et al., 2009), but it is less often identified as a source in outbreaks. However, two significant *Salmonella* outbreaks associated with ground beef have been reported in the US in the last 5 years (CDC, 2012; Schneider et al., 2011).

To prevent beef-related illnesses caused by bacterial pathogens and to reduce the economic costs of contamination it would be desirable if meat could be reliably decontaminated without sensory changes. Ionizing radiation is suitable for the decontamination of fresh meats as it can be used at bactericidal levels which do not substantially raise the internal temperature during treatment (Black & Jaczynski, 2006). Irradiation up to 10 kGray (kGy) absorbed dose has been established to pose no toxicological, microbiological or nutritional hazard by the Joint Expert Committee on

Food Irradiation of the Food and Agriculture Organization, the International Atomic Energy Authority, and the World Health Organization (WHO, 1981). Since 1999, the U.S. Food Safety and Inspection Service has approved the use of ionizing radiation to a maximum dose of 3.0 kGy for poultry, 4.5 kGy for refrigerated meat and 7.0 kGy for frozen meat (USDA, 1999a; 2008). Ionizing radiation has yet to be approved for the decontamination of fresh meat and poultry in Canada.

Ionizing radiation is of sufficient energy to produce charged or ionized atoms by removing electrons from their orbit. Ionizing radiation may be either in the form of electromagnetic waves such as gamma and X-rays which have high penetrating power or be particulate in nature, like alpha (He^{2+}) and beta (i.e. an electron) particles which weakly penetrate solid material (WHO, 2012). Ionizing radiation causes injury or death of bacterial cells by the destruction of essential macromolecules, such as DNA, RNA and proteins. Currently, cobalt-60 gamma sources, electron or e-beam generators, and X-ray accelerators are commonly used for food irradiation (USEPA, 2012).

An irradiation dose of 2.5 kGy can cause an 8 log reduction of *E. coli* O157:H7 or a 3 log reduction of salmonellae (Clavero et al., 1994). Such large reductions can yield complete inactivation of these pathogens in beef, where they are present at much lower levels. When irradiating large irregular surfaces or ground meats, high-penetration and high-energy radiation is used to ensure uniform product treatment (Arthur et al., 2005). However, irradiation of meat may lead to the development of off-odours and rancid flavours in a dose dependent manner, primarily due to lipid oxidation (Trindade et al., 2010; Alfaia et al., 2007). In healthy animals the internal tissues are usually sterile, but when meat is ground microorganism from the surface become internalised (USDA,

1999b). If the surface were decontaminated prior to grinding, the risk of pathogen re-distribution would be minimised. Therefore, to facilitate disinfection and avoid detectable organoleptic or nutritional changes, irradiation of beef trim before its fabrication into ground meat with a low-dose (≤1 kGy) and a low-penetrating (≤ 15 mm) e-beam holds promise (Arthur et al., 2005).

Currently there is little information available that characterizes or describes the radiation sensitivity of non-O157 VTEC and *Salmonella* strains on beef surfaces to 1 kGy e-beam treatment. Therefore, the main objective of this study was to determine the extent to which a 1 kGy dose could reduce the viability of mixtures of these organisms, grouped on the basis of radiation resistance, after their inoculation on fresh beef surfaces.

4.3. Materials and Methods

4.3.1. Bacterial strains

Thirty two strains of *E. coli* (19 different serotypes) that were positive for one or more verotoxin genes as determined by the PCR protocol of Paton & Paton (2003), and 6 strains of *Salmonella* were used in these tests. Strains were obtained from the Public Health Agency of Canada (Winnipeg, MB, Canada) and the Bureau of Microbial Hazards, Health Canada (Ottawa, ON, Canada) (Table 4.1). Cultures were maintained at -75 °C in glycerol-supplemented media and were streaked for growth on Brain Heart Infusion Agar (BHIA, Difco, Becton Dickinson and Co., Sparks, MD, USA), and then incubated at 37 °C for 24 to 48 h.

4.3.2. Inoculated meat studies with e-beam irradiation

To model the response of *E. coli* and *Salmonella* on the surface of carcasses to e-beam treatment, experiments were conducted on inoculated cuts of outside flat beef muscle. Choice of beef cuts was based on work by Geornaras et al. (2011). The meat was purchased the day before each trial from a retail butcher and frozen at -20 ^{o}C for 2 to 3 h to facilitate slicing. The meat pieces were cut with a meat slicer (Model 410, Hobart Manufacturing Co., Troy, OH, USA) into 1.2 cm thick strips which were cut into 5 ×5 cm squares (20 to 30 g/ piece) and stored at 4 °C in plastic autoclave bags.

4.3.3. Preparation of strains and meat inoculation

A number of different agar media were screened for suitability. Brilliant Green Agar with Sulfapyridine (BGS) (Neogen, Lansing, Michigan, USA), Xylose Lactose Dextrose Agar (XLD) (Becton Dickinson), Bismuth Sulfite Agar (Oxoid, Basingstoke, Hampshire, England) and Hectoen Enteric agar (Oxoid) were used for *Salmonella*; MacConkey Agar (Neogen)(MC), Sorbitol MacConkey Agar (Oxoid) with Cefixime-Tellurite (Dynal, Lake Success, NY, USA) (CTSMAC), Violet Red Bile Agar (VRB)(Oxoid), VRB with 1% Glucose (VRBG), Lactose Monensin Glucuronate Agar (LMG) (Neogen), Eosin Methylene Blue agar (EMB) (Oxoid) and VTEC agar (Gill et al., 2012) were used for *E. coli*. Plates were incubated 24 to 48 h at 35-37 ºC. Of these, LMG and BGS were used in trials because they permitted the best growth of all test strains of *E. coli* and *Salmonella*, respectively.

From a 10 mL overnight culture in BHI Broth, 0.2 mL was added to 200 mL BHI Broth and incubated at 35 to 37 °C for 24 h. Cultures were centrifuged for 10 min at 3752

xg (Avanti centrifuge J-26 XP, Beckman Coulter, Palo Alto, CA, USA). The supernatant was discarded and cell pellets were re-suspended in 200 mL 0.1 % peptone water. Cultures were diluted with 0.1% peptone to deliver 3 or 7 log CFU bacteria/g meat.

All 38 strains of bacteria were assigned to each of 8 groups (designated 1 to 8, Table 4.1) based on species and serotype and were mixed equally together to form 1 L of cell suspension for each individual group. Each cocktail of bacterial strains was added to a plastic autoclave bag containing 18 pieces of meat, and bags were manually massaged to facilitate even distribution of the bacteria. Preliminary tests revealed that *Salmonella* required a longer time for attachment to the meat tissue compared to *E. coli*, and so the *E. coli* groups were held for 20 to 30 sec and the *Salmonella* groups for 5 min to enable attachment. The inoculated meat samples were then allowed to drip on a perforated metal tray for 5 to 7 min and each piece was individually heat-sealed in a Winpak Deli *1 oxygen barrier bag measuring 7 x 9 cm (Winpak, Winnipeg, MB, Canada). For each experiment, 6 samples were prepared (i.e. three control and three treatment samples), each in triplicate. Inoculated control samples were stored at 4 °C and the rest of the samples were placed in an insulated container with ice packs and transported 90 min to Acsion Industries Inc. (Pinawa, MB, Canada) for 1 kGy e-beam irradiation. Samples were irradiated with a 10-MeV e-beam accelerator (Mevex, Stittsville, ON), and absorbed surface dose (0.79 to 1.14 kGy) was monitored with calorimeters (Risø Laboratories, Denmark) calibrated and traceable to the National Physical Laboratories, Teddington, Middlesex, UK. Samples were kept on frozen gel packs before irradiation, but the average temperatures immediately before and after irradiation were 12.5 ^{o}C and 19.4 ^{o}C, respectively. The e-beam application interval was < 30 min.

4.3.4. Enumeration of bacterial survivors

Irradiated meat samples were transported back to the laboratory and the number of survivors was enumerated without delay by plating on agar media. Additional enumeration was performed after 2 and 5 d storage at 4 °C.

Samples were placed in stomacher bags (Filtra-Bag, Fisher Scientific, Whitby, ON, Canada) and diluted 10-fold by weight using 0.1% peptone for control and BHI broth for treated samples. Treated samples in BHI were incubated at room temperature (25 °C) for 3 h to allow resuscitation of injured cells. Samples were then pummeled in a stomacher (BagMixer 400, Intersciences Inc., Markham, ON, Canada) for 2 min and diluted in 0.1 % peptone. *E. coli*-inoculated samples were plated on LMG agar to enumerate the total number of injured and uninjured cells and on LMG with 0.15% bile salts to enumerate uninjured cells. To enumerate *Salmonella*, BGS agar was used for uninjured and BGS overlaid with 7 mL BHI Agar for injured and uninjured cells (Wu et al., 2001). Samples inoculated at 3 log CFU/g were enumerated by passing 10 mL of the suspended sample through a Hydrophobic Grid Membrane filter (Neogen) which was then placed on the agar media described above. Agar plates were incubated at 35 ± 2°C for up to 48 h.

Because the broth tests suggested potential heterogeneity in resistance, strains in *E. coli* group 3 and *Salmonella* group 7 were individually exposed to 1 kGy on meat surfaces to identify the most resistant strains of these groups. Six pieces of meat (outside flat) were inoculated with 7 log CFU/g of each strain. The meat was e-beam irradiated as previously described and the treated meats were held at 4 °C overnight before enumeration could be done because of logistic constraints.

4.3.5. Comparison of treatments on lean and fatty tissue

The e-beam irradiation experiment was repeated using representative strains from within each serogroup showing greater resistance at 1 kGy. Four new groups were assembled: groups A to C contained *E. coli* strains and group D contained a mixture of three *Salmonella* serovars (Table 4.2). Lean tissue from the bottom of the inside round cuts and fatty tissue from the top of inside round muscle samples were inoculated, treated and analyzed as described with outside flat muscles

4.3.6. Statistical Analysis

All statistical analyses were performed with JMP® 8.0.1 (SAS Institute Inc.).The data means were compared using analysis of variance (ANOVA) and statistical differences between means were tested using Tukey's test with $\alpha = 0.05$.

4.4. Results and Discussion

4.4.1. Recovery media for irradiated cells

When each of the 8 bacterial groups from outside round meat trials were evaluated for survivors at both the low and high inoculum levels, significant differences between the numbers of survivors recovered on the selective and non-selective media were observed only for group 5 and 6 at high inoculum levels (data not shown). For the rest of the groups and with the low inoculum levels no significant difference between the two media were observed, indicating that incubation of the samples in BHI broth for 3 h at 25 °C was effective in promoting the recovery of any sub-lethally injured cells (Table 4.3).

4.4.2. Resistance of VTEC *E. coli* cocktails on meat

E-beam treatment of beef at 1 kGy significantly ($p < 0.05$) reduced the initial *E. coli* numbers in samples. For the low inoculum samples (3 log CFU/g), the highest number of survivors from the treated meat samples was observed for group 4 on day 0 (2.19 log CFU/g on selective agar). This was the lowest log reduction observed (<1.6 on day 0) among the 8 groups of inoculated organisms. For the rest of the *E. coli* groups, survivors were between 0 to 1 log CFU/g from day 0 to day 5 at 4 °C for the treated samples. For the high inoculum (7 log CFU/g) samples, groups 3 and 6 showed the greatest resistance, with survivors numbering from 3.75 to 4.13 log CFU/g from day 0 to day 5 at 4 °C. All other groups showed larger reductions of 4 to 6 log CFU/g. Group 3 was the most resistant culture mixture at 1 kGy (Table 4.3). Arthur et al. (2005) used a similar protocol to study the efficacy of low dose e-beams against *E. coli* O157:H7 on the carcass surface. Carcass tissues were inoculated at high (10^8 CFU/mL) and low levels (10^5 CFU/mL), irradiated at 1 kGy and stored at 4 °C for up to 120 h. The low inoculum-irradiated samples showed a reduction of 2.9 and 2.6 log CFU, after 48 h and 120 h storage, respectively. Irradiated samples with high inocula had a maximum reduction of 6.6 log CFU and 5.7 log CFU after 48 h and 120 h of storage, respectively. All but two of the irradiated samples were below the detection limit of 1 log/cm^2. In a study by Peirson et al. (2005), it was found that 1.1 kGy reduced the viability of a 5 strain *E. coli* O157:H7 cocktail in ground beef by ≤ 2.9 log CFU/g. The reduction obtained in the present work for the 4 strain cocktail of *E. coli* O157:H7 (group 1) was ≤ 5.81 log CFU/g on day 5 of storage at 4 °C. The greater reduction in the present work may have been related to the use of surface inoculation, since Peirson et al. (2005) mixed the inoculum within the

ground meat; in addition, the studies used different strains of *E. coli* O157:H7. The present results are similar to those reported by Arthur et al. (2005), even though the latter used only a single *E. coli* O157:H7 strain for their tests.

When the 7 strains of VTEC *E. coli* from group 3 were irradiated individually after inoculation on outside flat cuts, *E. coli* O85:H11 (03-3638) was observed to be the most resistant (4.47 log reduction), while 5 to 6 log reductions were observed among the rest of the strains. Since no survivors were recovered from the meat samples inoculated with low levels of group 6 organisms, this group was not investigated further.

4.4.3. Resistance of *Salmonella* cocktails on meat

Of all the organisms tested *Salmonella* in group 7 were the most resistant to irradiation at 1 kGy. Less than a 1 log reduction was observed in meat samples inoculated with 3 log CFU/g and a 2 log reduction was observed in meat inoculated with 7 log CFU/g. All 5 strains in this group were irradiated individually after inoculation on outside flat cuts. *Salmonella* Muenster and *S.* Derby were found to have the lowest resistance (> 3.80 log reduction) and *S.* Montevideo showed the highest resistance (1.94 log reduction). Group 8 (*S.* Newport) was more susceptible to irradiation, showing 3 log reductions at both low and high inoculum levels.

4.4.4. Influence of fat and post-irradiation growth

Based on D_{10} values from the preliminary X-ray study with individual cultures (Chapter 3) and the inoculated outside flat meat trials, strains with slightly higher resistance at ≤ 1 kGy were selected to represent strains from each *E. coli* serotype plus three serovars of *Salmonella* (Table 4.2). When e-beam irradiation was repeated at 1 kGy

using three different fresh beef muscles (outside flat, top and bottom of the inside round) it was found that the type of muscle did not significantly affect the number of survivors following irradiation, and the results were combined (Table 4.4). Although the thin layer of fat on one side of the inside round cuts used here did not influence bacterial survival, greater reductions of *E. coli* O157:H7 and non-O157 VTEC on fatty beef tissue than on lean samples were reported following non-irradiated decontamination with 5% lactic acid at 55 °C (Geornaras et al., 2011). Although refrigerated storage at 4 °C for up to 5 d did not have a significant effect on the recovery of survivors, the numbers recovered were slightly lower for some groups of bacteria on day 5. This was taken to indicate that irradiation treatment did not increase the risk of their growth during storage.

4.4.5. Resistance of *Salmonella* and VTEC *E. coli* on fresh beef

The greatest reduction among the *E. coli* groups studied was observed in group A with an average log reduction of 4.5 log CFU/g. The group B strain (O157:H7 EO122) was reduced by 4.1 log CFU/g and group C strains by 4 log CFU/g. The lowest reduction (< 2 log) was observed with *Salmonella* in group D (Table 4.5). The D_{10} value can be used for comparing radiation resistance (Dion et al., 1994), and where reported, they show that *Salmonella* is more radiation-resistant that *E. coli* O157:H7. Clavero et al. (1994) found D_{10} values for *E. coli* O157:H7 and salmonellae on raw ground beef patties ranged from 0.241 to 0.307 kGy and 0.618 to 0.800 kGy, respectively. Thayer et al. (1995) reported similar D_{10} values on beef for *E. coli* O157:H7 (0.3 kGy) and *Salmonella spp*. (0.7 kGy). In the present study the reductions in viability following irradiation suggest that higher D_{10} values than those reported by others were likely; however, the

difference in relative resistance of the two genera were similar in that *Salmonella* spp. were about 2-fold more resistant than VTEC *E. coli*.

Data indicate that differences in radiation sensitivity exist not only among species but also between strains (serovars, serotypes) of the same species (unpublished this laboratory, Diehl, 1990). Clavero et al. (1994) suggested that cell size and structural arrangement of DNA within the cell contribute to differences in sensitivities to irradiation. In non-pigmented bacteria radiation-resistance of species is primarily influenced by the ability of the organism to repair radiation-induced damage (Kapp & Smith, 1970). However, the different resistances observed between different strains when they are separately irradiated may be due in part to differences in physiological activity existing at the moment of treatment, suggesting that it is also influenced by the metabolic phase of the microorganism itself (Dion et al., 1994).

Even though the level of reduction observed for the most resistant organisms tested in the present study was only about 2 log CFU/g, this should be sufficient to eliminate enteric pathogens present on carcass surfaces. The VTEC pathogens can be considered to be a subset of *E. coli* and coliform populations on carcass sides. Enumeration of these floras on washed carcass sides in two studies found mean *E. coli* numbers of 0.1004 and 0.1003 log CFU/100 cm^2 in two studies (n=25) and coliform numbers of 0.1012 and 0.1164 log CFU/100 cm^2 (Gill et al., 2003; Gill & Landers, 2003). Further, Luchansky et al. (2012) cited earlier work reporting the typical occurrence of pathogenic *E. coli* on beef subprimal surfaces to be < 0.375 CFU/cm^2. Therefore, at such low contamination levels, a dose of 1 kGy e-beam irradiation would be more than adequate to provide a large margin of safety from contamination of fresh beef by the pathogens tested here.

When intact meat surfaces are treated with e-beam radiation at low doses, because of its limited penetration only a small proportion of the treated surface meat would be present in mixed ground beef made from it. It was believed that because of this feature no differences were observed by a sensory panel which evaluated ground beef containing 10% irradiated meat, but sensory differences in aroma and flavour were detected with patties containing > 25% irradiated meat (Arthur et al., 2005). In another study a dose of ≤ 3.0 kGy did not produce detectable changes in grilled patties prepared from irradiated ground beef and only slight changes were detected with a dose of 4.5 kGy (Wheeler et al., 1999). Initially consumers may be apprehensive of purchasing irradiated meat products, but respond favourably once additional information is provided (Terry & Tabour, 1988). Therefore, it is highly probable that at 1 kGy minimal changes in consumer acceptance are likely, based on meat sensory attributes. Use of e-beam irradiation has shown promising results here and it would be an important asset for the meat industry to assure consumer safety.

Table 4.1: Organisms and groups of strains used for meat inoculation.

Organism	Serotype	Strain No.	Group
Escherichia coli	O157:H7	1934	1
E. coli	O157:H7	1931	1
E. coli	O157:H7	161-84	1
E. coli	O157:H7	CO283	1
E. coli	O111:NM	98-8338	2
E. coli	O111:NM	CFS3	2
E. coli	O111:NM	CFS4	2
E. coli	O177:NM	03-3974	2
E. coli	O5:NM	03-2835	2
E. coli	O145:NM	03-4699	2
E. coli	O26:H11	00-3941	3
E. coli	O26:H11	01-5870	3
E. coli	O26:H11	02-6738	3
E. coli	O85:H11	03-3638	3
E. coli	O121:H19	00-5288	3
E. coli	O121:H19	03-2642	3
E. coli	O121:H19	03-2832	3
E. coli	O145:H2	75-83	4
E. coli	O45:H2	05-6545	4
E. coli	O45:H2	04-2445	4
E. coli	O103:H2	99-2076	4
E. coli	O103:H2	04-2446	4
E. coli	O103:H2	01-6102	4
E. coli	O6:H34	03-5166	5
E. coli	O117:H7	02-4495	5
E. coli	O165:H25	00-4540	5
E. coli	O55:H7	05-0376	5
E. coli	O113:H21	93-0016	5
E. coli	O91:H21	85-489	5
E. coli	O157:H7	EO122	6
E. coli	O145:NM	04-7099	6
E. coli	O115:H18	03-3645	6
Salmonella Montevideo		02-4092	7
S. Derby		MM	7
S. Enteritidis		CRIFS	7
S. Muenster		184	7
S. Typhimurium		1193	7
S. Newport		1194	8

Table 4.2: Bacterial groups containing strains with elevated irradiation resistance used as mixed inocula on three meat cuts treated with 1 kGy e-beam irradiation.

Group	Organism	Serotype	Strain No.
A	*Escherichia coli*	O111:NM	98-8338
A	*E. coli*	O145:NM	04-7099
A	*E. coli*	O103:H2	04-2446
A	*E. coli*	O45:H2	05-6545
B	*E. coli*	O157:H7	EO122
C	*E. coli*	O115:H18	03-3645
C	*E. coli*	O26:H11	00-3941
C	*E. coli*	O121:H19	03-2642
C	*E. coli*	O85:H11	03-3638
D	*Salmonella* Montevideo		02-4092
D	*S.* Enteritidis		CRIFS
D	*S.* Typhimurium		1193

Table 4.3: Survival (log CFU/g) of VTEC *E. coli* and *Salmonella* on meat inoculated at 3 or 7 log CFU/g, e-beam irradiated at 1 kGy and stored up to 5 days at 4 °C.

		High Inoculum Samples			Low Inoculum Samples		
	Treatment	Day 0	Day 2	Day 5	Day 0	Day 2	Day 5
Group 1[a]	Control	7.65	7.53	7.83	3.78	3.74	3.87
	Selective agar	2.30	2.20	1.15	0.94	<DL[b]	<DL
	Non-selective agar	2.10	2.98	2.02	1.51	<DL	<DL
Group 2	Control	7.58	7.33	7.84	3.61	3.21	3.75
	Selective agar	3.15	2.08	3.30	<DL	<DL	0.77
	Non-selective agar	3.17	2.09	3.24	<DL	<DL	1.50
Group 3	Control	7.61	7.43	7.51	3.96	4.44	3.96
	Selective agar	3.75	3.12	3.14	<DL	<DL	<DL
	Non-selective agar	3.78	3.16	3.44	<DL	<DL	<DL
Group 4	Control	7.6	7.65	8.05	3.77	3.47	3.75
	Selective agar	2.61	2.88	3.00	2.19	<DL	<DL
	Non-selective agar	2.31	2.91	3.17	2.08	<DL	<DL
Group 5	Control	7.52	7.84	7.63	3.64	3.73	3.63
	Selective agar	1.80	1.80	3.11	0.88	<DL	<DL
	Non-selective agar	3.06	3.12	3.18	0.92	<DL	<DL
Group 6	Control	7.45	7.61	7.69	3.76	3.74	3.89
	Selective agar	3.57	3.53	3.98	<DL	<DL	<DL
	Non-selective agar	3.68	3.99	4.13	<DL	<DL	<DL
Group 7	Control	7.38	7.47	7.48	3.82	3.93	3.47
	Selective agar	5.05	5.45	6.18	3.69	3.69	<DL
	Non-selective agar	5.32	5.48	6.29	3.31	3.41	<DL
Group 8	Control	7.02	7.22	7.00	3.98	4.37	3.97
	Selective agar	4.60	4.49	4.63	<DL	1.07	1.2
	Non-selective agar	4.24	4.10	4.57	<DL	<DL	2.33

[a] Strains in each group are identified in Table 4.1. *E. coli* in groups 1 to 6 were recovered on selective (LMG+ Bile) and non-selective (LMG) agars. *Salmonella* in groups 7 and 8 were recovered on selective (BGS) and non-selective (BGS+BHI) agars. Data are means of 3 replicates.

[b] Detection limit (DL) was 0.82 log CFU/g.

Table 4.4: Means of log CFU/g surviving *E. coli* and *Salmonella* on e-beam irradiated (1 kGy) outside flats, top inside round, and bottom inside round cuts of fresh beef stored up to 5 days at 4 °C.

	Treatment	Day 0	Day 2	Day 5
Group A[a]	Control	7.54	7.46	7.47
	Selective agar	2.91	2.93	2.90
	Non-selective agar	2.61	3.09	3.21
Group B	Control	7.63	7.61	7.58
	Selective agar	3.45	3.32	3.55
	Non-selective agar	3.49	3.54	3.75
Group C	Control	7.45	7.34	7.33
	Selective agar	3.27	3.30	3.50
	Non-selective agar	3.45	3.39	3.64
Group D	Control	7.41	7.31	7.20
	Selective agar	5.44	5.46	5.24
	Non-selective agar	5.45	5.50	5.32

[a] Strains in each group are identified in Table 2. *E. coli* in groups A to C were recovered on selective (LMG+ Bile) and non-selective (LMG) agars. *Salmonella* in group D were recovered on selective (BGS) and non-selective (BGS+BHI) agars.
Data are given as means of 9 replicates.

Table 4.5: Average log CFU/g of surviving *E. coli* and *Salmonella* on e-beam irradiated (1 kGy) outside flats, top inside round, and bottom inside round cuts of fresh beef from all 5 days of storage at 4 °C.

Treatment	Group A[a]	Group B	Group C	Group D
Control	7.48 b	7.60 a	7.37 c	7.30 c
Selective agar	2.90 c	3.43 b	3.35 b	5.38 a
Non-selective agar	2.98 c	3.59 b	3.49 b	5.42 a

[a] Strains in each group are identified in Table 2. *E. coli* in groups A to C were recovered on selective (LMG+ Bile) and non-selective (LMG) agars. *Salmonella* in group D were recovered on selective (BGS) and non-selective (BGS+BHI) agars.
Values are a mean log CFU/g (n=18). Data in columns followed by a different letter are significantly different.

CHAPTER 5

Effect of Low-Dose Electron Beam Irradiation on Quality of Ground Beef Patties and Raw, Intact Carcass Muscle Pieces.

5.1. Abstract

The objectives of this study were to determine the effects of a low-dose (≤1 kGy), low-penetration electron beam on the sensory qualities of (1) raw muscle pieces of beef and (2) cooked ground beef patties. Outside flat, inside round, brisket and sirloin muscle pieces were used as models to demonstrate the effect of irradiation on raw beef odour and colour, as evaluated by a trained panel. Ground beef patties were also evaluated by a trained panel, but for tenderness, juiciness, beef flavour, and aroma at 10 %, 20% and 30% levels of fat, containing 0% (control), 10%, 20%, 50% and 100% irradiated meat. With whole muscle pieces, the colour of controls appeared more red ($p < 0.05$) than irradiated muscles, however, both control and treatments showed a gradual deterioration in colour over 14 d aerobic storage at 4 °C. Off-aroma intensity of both control and treatments increased with storage time, but by day 14, the treated muscles showed significantly ($p < 0.05$) less off-aroma than the controls, presumably as a result of a lower microbial load. It was found that a 1kGy absorbed dose had minimal effects on the sensory properties of intact beef muscle pieces. Irradiation did not have a significant effect ($p > 0.05$) on any of the sensory attributes of the patties. Low dose irradiation of beef trim to formulate ground beef appears to be a viable alternative processing approach that does not affect product quality.

5.2. Practical Application

Sensory panels could not detect organoleptic differences between 1 kGy irradiated and non-irradiated portions of intact beef muscle or cooked ground beef patties made from the treated meat. Data obtained support the concept that that low dose irradiation of beef trim may be a workable solution for the contamination of ground beef by toxigenic (STEC) *E. coli*.

5.3. Introduction

Food irradiation is the most extensively studied of all technologies used to process food and relies upon exposure of products to ionizing radiation of sufficient energy to produce charged atoms. Irradiation causes damage or death to microorganisms by destroying vital macromolecules such as DNA, RNA and proteins, thereby disrupting normal cell function. The most common sources of irradiation used for food are electron or e-beam accelerators, X-rays and cobalt-60 gamma sources (WHO, 2012; USEPA, 2012; FoodSciAust, 2006). The former equipment is by far the most commonly used in most current food applications, but while treatment usually improves product shelf-life, it can negatively affect organoleptic properties in a dose dependent manner.

At source energy and dose levels permitted, irradiation cannot cause radioactive decay of foods; however, chemical changes can occur due to the formation of ionization-catalyzed radiolytic products. These radiolytic products pose no known health hazard and the hundreds of different volatile compounds that are detected in irradiated beef can form naturally in non-irradiated beef during cooking, mostly due to reactions with lipid (Chen et al., 2002). In 1981, the Joint Expert Committee on the Wholesomeness of Irradiated

Food (JECFI) concluded that food commodities treated up to an overall average dose of 10 kGy were safe for human consumption since they posed no toxicological hazard or microbiological concern, and were nutritionally adequate (WHO, 1981). In 1997 the JECFI drew the same conclusion for foods irradiated at 25 to 60 kGy (Farkas & Mohácsi-Farkas, 2011). The use of ionizing radiation to a maximum dose of 3.0 kGy for poultry, 4.5 kGy for refrigerated meat and 7.0 kGy for frozen meat has been approved by the U.S. Food Safety and Inspection Service (USDA, 1999; 2008).

Slow progress to the adoption of food irradiation is mainly due to psychological and political factors. Obstacles to its use include logistic bottlenecks (its availability at production sites), cost of the technology, and concern by industry decision-makers about public perception of the process. In many countries there is a trend that as consumers become more informed, the process becomes acceptable. To maintain this growing support it then becomes important to ensure that radiolytic products which might form in treated products are minimized by controlling radiation dose, temperature, product moisture, and package gas atmosphere (WHO, 1981). Studies have shown with meat products that doses of ≤ 1 kGy cause minimal sensory changes. However, the detection of off-odours and off-flavours increased at doses ≥ 2 kGy (Thayer, 1993). Studies have also shown that a dose as low as 1kGy can reduce the numbers of *E. coli* O157:H7 by 3 to 4 log cfu/g (Arthur et al., 2005; Peirson et al., 2005). However, the irregular geometry of the animal carcass means that distances between inner and outer surfaces (where contamination is localized) are highly variable and make it difficult to dependably deliver uniform levels of irradiation sufficient to eliminate pathogens without affecting the sensory characteristics of treated meat. A suitable alternative to eliminate toxigenic *E.*

coli contamination of ground beef might be the irradiation of carcass trim before it is ground to form hamburger.

The purpose of this study was to explore whether undesirable sensory changes could be detected following irradiation of beef pieces representing trim and ground beef made from it. The main objectives were to determine whether an absorbed dose of 1 kGy e-beam radiation has detectable effects upon (1) the appearance and aroma of selected carcass muscles with and without fat following irradiation and (2) the sensory characteristics of cooked beef patties containing different levels of fat and differing amounts of irradiated beef trim pieces.

5.4. Materials and Methods

5.4.1. Irradiation

All samples were irradiated with a 10-MeV E-beam accelerator (Mevex, Stittsville, ON) at Acsion Industries Inc. (Pinawa, MB, Canada). The absorbed dose was monitored with calorimeters (Risø Laboratories, Denmark) calibrated and traceable to the National Physical Laboratories, Middlesex, UK. The absorbed surface dose ranged from 0.97 to 1.11 kGy at a temperature of 22 °C.

5.4.2. Irradiated Raw Beef Surface Muscles

5.4.2.1. Preparation of beef muscles

Four beef muscles (outside flat, inside round, sirloin and brisket) were sliced to yield pieces 1.5 cm thick. For each muscle, slices measuring 5 × 5 cm were cut and divided into 4 lots, two lots containing slices with fat and two without fat were vacuum-packaged

in Winpak Deli *1 oxygen barrier bags measuring 7 x 9 cm and kept frozen at -20 °C. Each subsequent day, two lots of each muscle type with and without fat were transported in an insulated container with ice packs to Acsion Industries Inc. for 1 kGy e-beam irradiation. The time required for transport was 90 min and the e-beam application interval was < 30 min.

5.4.2.2. Sensory evaluation

Twelve students recruited from the University of Manitoba were trained through 4 sessions for 1 h each. Beef muscle pieces were evaluated by means of a 9-point scale for off-aroma (9 = none; 1= extremely intense) and colour (9 = extremely or 100% red; 1 = extremely or 100% brown). Standard products were selected that represented the definition for each attribute (Table 5.1). Panellists were trained to identify specific off-flavour and off-aroma descriptors and results were discussed among the group to minimize variation. Muscle pieces were evaluated at 1, 2 and 14 d storage at 4 °C after irradiation treatment. Picture colour standards were provided during experimental sessions for comparison. Two sessions were held each day, with 8 samples in each session from two different muscle types, with and without fat. Samples were identified using 3-digit codes and were served to each panelist in random order. Samples were removed from sealed packages 30 min before tests and presented in transparent plastic containers placed on a white table top. Evaluation was done in a non-partitioned room under cool white fluorescent light at an intensity of 150 lux, as measured with a LI-185B photometer (LI-Cor Inc., Lincoln, NE, US). The tests were conducted in 3 trials involving 18 sessions. Data were analyzed using 2-way ANOVA and Tukey's test was used to determine mean treatment differences when significant ($p < 0.05$) with JMP® 8.0.1 (SAS

Institute Inc.).

5.4.3. Irradiated Ground Beef Patties

5.4.3.1. Formulation of ground beef patties

Extra lean outside flat beef muscles (about 95% lean) and fat trim from the hind quarter of the carcass were mechanically sliced (Model 410, Hobart Manufacturing Co., Troy, OH, USA) into pieces ≤ 1.5 cm thick. These were vacuum-sealed in Winpak Deli *1 oxygen barrier bags measuring 7 x 9 cm (Winpak, Winnipeg, MB, Canada) and kept frozen at -20 °C. One half the lot was transported the following day in an insulated container with ice packs for 1 kGy e-beam irradiation.

The treated and the untreated lean muscles and fat trim were ground separately through the 0.3 cm plate of a 28.4 L floor mixer/grinder (Hobart) and combined to yield 3 target fat/lean meat combinations (10%/90%, 20%/80% and 30%/70%). Within each fat/lean combination, 5 groups of patties each containing 0, 10, 20, 50, and 100% irradiated meat/fat were prepared by blending irradiated with non-irradiated meat and fat to yield 1 kg batches of each type. The actual levels of fat were determined after freeze-drying at -60 °C for 72 h by Soxhlet hexane extraction (Table 5.2). Ground beef patties, each weighing 113 g were made using a plastic kitchen meat pattie form and were vacuum packaged in Winpak Deli *1 oxygen barrier bags measuring 7 x 9 cm and kept frozen at -20 °C for 10 d.

5.4.3.2. Cooking

Patties were thawed at 4 °C for 18 h and cooked on a preheated indoor grill (model

3600, Hamilton Beach, Picton, ON, Canada) at a temperature of 195 °C. Temperatures of the patties were checked with an Accu-temp digital meat thermometer with probe (Springfield Instruments, Montreal, QC, Canada) until 71^{o}C was reached to ensure safety. Groups of 5 patties were cooked on the grill for a total of 7 min, first using 3 min on each side, followed by 30 s again on each side. After cooking, patties were blotted on paper towel to remove surface grease. Each patty was portioned into 8 equally-sized wedges (16 wedges per sample) and were immediately served to panelists.

5.4.3.3. Sensory evaluation

Ground beef patties were evaluated by 12 trained panelists using a nine-point scale for tenderness (9 = extremely tender; 1 = extremely tough), juiciness (9 = extremely juicy; 1 = extremely dry), beef flavour (9 = extremely intense; 1 = none), off flavour (9 = none; 1 = extremely intense), beef aroma (9 = extremely intense; 1 = none), and off-aroma (9 = none; 1 = extremely intense) through 7 sessions for 1 h each. Panellists were trained to identify specific off-aroma and off-flavour descriptors associated with irradiated beef. Various samples were smelled and tasted to demonstrate the range of intensities. During training sessions, results were discussed among the group to minimize variation. Standard products were selected that represented the definition for each attribute (Table 5.3). Apple juice, unsalted crackers and water were used to cleanse the palate before each session and between samples. Three trials of each experiment involving 9 sessions were conducted. In each session all 5 irradiation treatments were evaluated at each fixed level of fat. Each sample was given a 3-digit numerical code and presented in random order. Ground beef sensory data were analyzed by one-way ANOVA and Tukey's test was used to determine mean treatment differences when

significant ($p < 0.05$) using JMP® 8.0.1 (SAS Institute Inc.).

5.5. Results and Discussion

A number of studies examining the effect of irradiation on meat quality have shown that discolouration, rancid off-odours and flavours can result from the oxidative action of radiolytic products on lipid (Murano, 1995). For example, irradiation of meat in the presence of oxygen may lead to bleaching discolouration caused by the development of ozone, which is a strong oxidizing agent (Olson, 1998). The development of these undesirable traits is dependent on a combination of factors such as the radiation dose, dose rate, packaging conditions, and temperature (Thayer, 1990). Generally, the development of unsatisfactory characteristics increases at higher radiation doses. Therefore, it is essential that doses higher than necessary to achieve the desired results not be used (WHO, 1981).

Since the effect of irradiation may be different in raw and cooked meat, the colour and off-aroma of raw beef muscles were analysed. The interaction between treatment and storage was not significant ($p > 0.05$). Colour was significantly affected ($p <0.05$) by the irradiation treatment, where controls samples showed a significantly ($p < 0.05$) higher (more red) score compared to irradiated samples, which appeared more brown (Table 5.4). Storage at 4 °C also had a significant affect ($p < 0.05$) on colour. Decreasing colour ratings for both control and irradiated samples were observed with increasing days in storage.

Metmyoglobin-reducing capacity of fresh meat is essential for the meat to retain its capacity to bloom to a red colour following its removal from vacuum packages (Benedict

et al., 1975; Li et al., 2012). Metmyoglobin is the pigment responsible for the characteristic brown colour of meat as it deteriorates during refrigerated storage (Mancini & Hunt, 2005). Although vacuum packaging of fresh beef is expected to delay browning of meat, many panellists observed the presence of a peripheral brown ring in both control and treated samples, indicating irreversible browning which typically occurs with meat that is near the end of its refrigerated shelf-life. The presence of exudates within the wrinkles of the packaging film can foster the growth of spoilage microorganisms, further reducing the colour stability of beef (Vasques et al., 2004). As the rate of discolouration varies among muscle types (Hood, 1980), it was not surprising that the type of muscle had a significant affect ($p < 0.05$) on colour (data not shown). It is possible that this difference was enhanced during storage as the meat was likely aged to varying lengths when bought from the supplier, and changes were not a direct result of irradiation treatment.

In a review, Nam & Ahn (2003) suggested that the mechanism of colour change in irradiated meat would be similar to that in non-irradiated meat. Nanke et al. (1998) observed that the effect of irradiation on colour of vacuum-packaged meat differed between different types of meat and was dose-dependent. This difference between meat types was supported by Nam & Ahn (2002), who noted that different mechanisms and pigments were responsible for irradiation-induced colour changes in turkey meat; however, in beef the mechanism is still undetermined. Certainly, colour is an attribute that is greatly affected by the packaging conditions (Luchsinger et al., 1996). Irradiated, vacuum-packaged pork and turkey appeared to be redder and the colour was stable over time, whereas irradiated vacuum-packaged beef showed browning at doses as low as 1.5

kGy (Nanke et al., 1998), which is consistent the observations made in the present study. However, this is in contrast with several other studies, which found that irradiating meat in the absence of oxygen resulted in a bright red colour similar to oxymyoglobin (Luchsinger et al., 1996; Nanke et al., 1998). Luchsinger et al. (1997) demonstrated that vacuum-packaged steaks irradiated at 3.5 kGy appeared more red than non-irradiated steaks.

Interestingly, for the off-aroma attribute, although both irradiation treatment and storage had a significant affect ($p< 0.05$), the interaction of the two was also significant ($p< 0.05$). Therefore, the results were further analysed in terms of the interaction (Table 5.5). The control and treated samples showed no significant difference in off-aroma ratings on day 1 and day 2 of storage. However, the magnitude of the difference between the control and treated muscles was much larger on day 14, where the treated samples displayed a significantly ($p< 0.05$) higher off-aroma rating compared to the control samples. Overall, the general decline in off-aroma rating for both treatments indicate spoilage induced off-aroma. However, better off-aroma rating of the irradiated samples by day 14 of storage suggests that the treated samples contained lower numbers of viable spoilage microorganisms, which might have delayed their spoilage compared to the control samples (Murano et al., 1998). Off-aroma ratings were not significantly affected ($p> 0.05$) by the type of muscle (data not shown).

The presence or absence of fat showed no significant ($p > 0.05$) affect on the colour or off-aroma attribute for all 4 muscles (data not shown). However, the colour of samples with fat appeared to be more red than the samples without fat irrespective of treatment. It is possible since differences were independent of irradiation, that the

perception of red colour was affected by its contrast with the slightly yellowish coloured fat, causing the colour of lean tissue adjacent to fat to appear to be more brightly red than when lean tissue was viewed alone.

When irradiated ground beef patties containing 3 levels of fat and 5 concentrations of irradiated meat were examined for sensory changes, the interaction of treatment and fat was not significant ($p > 0.05$) for any trait. The effect of treatment (irradiated meat %) for each of the sensory attributes was also not significant ($p > 0.05$) (Table 5.6). Fat affected only juiciness ratings, where patties made with 30 % fat were significantly ($p < 0.05$) juicier than patties made with 10% fat.

A few of the panel members identified off-flavour and off-aroma characteristics described as burnt, bitter and sour in 50% and 100% irradiated samples regardless of fat content. This did not significantly affect the overall ratings of attributes. It should be considered that when ground beef is prepared from a surface-irradiated carcass, only a small portion (maximum 10%) of the final mix would contain irradiated tissue (Arthur et al., 2005). In the latter and present studies, 100%-irradiated treatments were included as positive controls. Hence it is highly unlikely that the mild off-flavours observed in the present study would be detectable in commercial products. In fact, off-odours from irradiated meat appear to be reduced or completely disappear upon being cooked, indicating that the problem might only concern raw meat (Olson, 1998). Although the sensory panel did not assess the colour of ground beef patties, informal colour measurements were noted before and after cooking. Panelists were asked to comment on any exceptional traits and no colour differences were observed in either instance.

The effect of irradiation on the sensory qualities of meat has been studied using various meat products, and the results have been both positive and negative. Arthur et al. (2005) assessed the impact of a 1 kGy dose of e-beam irradiation on rough-cut flank steaks representing whole muscles with 5, 10, 25, 50 and 75% irradiated beef and ground beef patties containing 0, 5, 10, 25, 50 and 100% surface-irradiated beef muscles. Sensory attributes such as beef-aroma, off-aroma, tenderness, juiciness, beef flavour and off-flavour were measured and irradiation did not affect any of the attributes studied. With ground beef, no differences were detected among the control, 5 or 10% treated samples. However, in contrast with the present study, the 100% irradiated samples received less favorable beef aroma and beef flavour intensity ratings. In another study, Wheeler et al. (1999) used trained sensory and consumer panels to evaluate vacuum-packaged frozen ground beef patties irradiated at 0, 3 and 4.5 kGy. The trained panel detected a more intense beef aroma and less off-aroma among the control patties; however, no differences were detected between the 3 kGy and 4.5 kGy treated samples. On the other hand, the consumer panel rated hamburgers treated at all levels as “fair”. It is notable that the latter panel gave a lower taste score to hamburgers treated at 4.5 kGy than to those treated at 0 or 3 kGy.

In the present study, the effect of storage on cooked ground beef patties was not evaluated, but studies have shown that trained panelists could not detect differences between patties treated at 1, 3, and 5 kGy and those non-irradiated, which were examined during 6 weeks storage at -18 °C. Differences could be detected only at a dose of 7 kGy after 5 weeks of storage. It appears that odour problems might be greater with irradiated raw meat, but in a study by Fu et al. (1995) at dose levels of 0, 0.6 and 1.5 kGy with meat

then stored for 7 d at 7 °C, no odour differences were detected by an untrained panel. Although at higher doses sensory changes may be noticeable, with 1 kGy irradiation, sensory changes in fresh beef were minimal or absent.

Table 5.1: Attribute definitions and standards used for analysis of raw carcass muscles.

Attribute	Description	Standard (High-end of scale)	Standard (Low-end of scale)
Colour	External surface colour	Picture standards equivalent to #5 Bright cherry red Munsell colour chips for beef retail colour	Picture standards equivalent to #8 extremely dark red Munsell colour chips for beef retail colour
Off-Aroma	Any aroma not evaluated as beef	None	Rancid: Month old heat-treated (85 °C for 48 h) raw unsalted peanuts (PC brand, Superstore). Cardboardy: 20 X 2.5 cm^2 pieces of cardboard cooked in 500 ml boiling water for 5 min. Grainy: 50 gm feed barley cooked in 600 ml boiling water for 8 min.

Table 5.2: Formulation of irradiated ground beef patties and actual % fat.

Target % Fat	Target Irradiated Content %	Untreated Fat (grams)	Untreated Lean Meat (grams)	Treated Fat (grams)	Treated Lean Meat (grams)	Actual % Fat
10	0	50	950	0	0	12.43
	10	45	875	5	95	
	20	40	760	10	190	
	50	25	475	25	475	
	100	0	0	50	950	
20	0	150	850	0	0	21.93
	10	135	765	15	85	
	20	120	680	30	170	
	50	75	425	75	425	
	100	0	0	150	850	
30	0	250	750	0	0	29.48
	10	225	675	25	75	
	20	200	600	50	150	
	50	125	375	125	375	
	100	0	0	250	750	

Table 5.3: Attribute definitions and standards used for analysis of cooked ground beef patty.

Attribute	Description	Standard (High-end of scale)	Standard (Low-end of scale)
Tenderness	Ease of biting through half of the sample on the first bite	2.5 cm cubes of cream cheese (Selections brand, IGA)	Raw carrots (produce section, IGA)
Juiciness	Amount of moisture present after 3-5 chews	Canned pineapple chunks (Dole)	Black liquorice (bulk section, Superstore)
Beef Flavour	Characteristic beef flavour intensity after sample has been completely chewed	Warm, freshly cooked regular ground beef patty (IGA)	None
Beef Aroma	Characteristic smell associated with cooked beef after three short sniffs	Warm, freshly cooked regular ground beef patty (IGA)	None
Off-Aroma	Any aroma not evaluated as beef	None	Rancid: Month old heat-treated (85 °C for 48 h) raw unsalted peanuts (PC brand, Superstore). Cardboardy: 20 X 2.5 cm^2 pieces of cardboard cooked in 500 ml boiling water for 5 min. Grainy: 50 gm feed barley cooked in 600 ml boiling water for 8 min.
Off-Flavour	Any flavour not evaluated as beef after the sample has been completely chewed	None	Sour: 1% citric acid (Life brand, Shopper's drug mart). Bitter: (0.3% caffeine (Life brand, shopper's drug mart)

Table 5.4: Effect of irradiation and storage at 4 °C on trained sensory panel ratings[a] of intact muscle cut colour.

Main Effect	
Treatment	Colour
Control	6.70 a
Test	6.00 b
SEM	0.06
P value	0.0001
Storage day	
1	6.93 a
2	6.31 b
14	5.83 c
SEM	0.07
P value	0.0001

[a]Ratings for the colour attribute ranged from 9 (extremely or 100% red) to 1 (extremely or 100% brown). Data in rows within each muscle group not followed by the same letter are significantly different.

Within main effect, means in the same column not followed by a common letter are significantly different.

Table 5.5: Effect of interaction of irradiation and storage at 4 °C on off-aroma evaluated by trained sensory panel ratings[a] of intact muscle pieces.

	Treatment	
Storage time	Control	Test
1	8.23 a	7.84 a
2	8.16 a	8.03 a
14	4.67 c	6.36 b
Pooled SEM	0.11	
P value	<0.0001	

[a]Ratings for the off-aroma attribute ranged from 9 (none) to 1 (extremely intense). Data not followed by the same letter are significantly different.

Table 5.6: Effect of amount of irradiated meat and fat % on trained sensory panel ratings[a] of ground beef patties

Main Effect	Beef Aroma	Off-Aroma	Tenderness	Juiciness	Beef Flavour	Off-Flavour
Treatment						
0	7.28 a	8.69 a	6.17 a	5.94 a	7.26 a	8.60 a
10	6.96 a	8.64 a	6.08 a	6.24 a	7.05 a	8.45 a
20	7.15 a	8.73 a	6.01 a	5.90 a	7.13 a	8.72 a
50	7.53 a	8.70 a	6.15 a	5.91 a	7.41 a	8.60 a
100	7.44 a	8.74 a	6.38 a	5.69 a	7.38 a	8.46 a
SEM	0.15	0.06	0.17	0.14	0.16	0.08
P Value	0.05	0.84	0.65	0.12	0.46	0.14
Fat (%)						
10	7.25 a	8.67 a	6.21 a	5.74 b	7.13 a	8.44 a
20	7.24 a	8.72 a	6.18 a	5.94 ab	7.41 a	8.65 a
30	7.33 a	8.71 a	6.07 a	6.13 a	7.20 a	8.61 a
SEM	0.11	0.04	0.13	0.11	0.12	0.06
P value	0.82	0.77	0.75	0.05	0.25	0.06

[a]Ratings for attributes ranged from 9 to 1; tenderness (9=extremely tender; 1= extremely tough), juiciness (9=extremely juicy; 1=extremely dry), beef flavour (9= extremely intense; 1= none), off flavour (9=none; 1= extremely intense), beef aroma (9= extremely intense; 1= none), and off-aroma (9=none; 1= extremely intense).

Within main effect, means in the same column that do not share a common letter are significantly different.

CHAPTER 6

6. Conclusions

Decimal reduction dose (D) values estimated in phosphate buffer showed that *E. coli* O157:H7 and non-O157 (VTEC) *E. coli* had similar radiation sensitivity, indicating that these unconventional pathogens may respond to irradiation processing in a similar fashion. However, *Salmonella* appeared to have higher resistance to the X-ray treatment.

Results from the meat trials also confirmed that non-O157 VTEC were no more radiation resistant than O157 VTEC with at least a 4 log reduction for all groups of VTEC tested. Again, *Salmonella* strains were more radiation resistant than VTEC with only a < 2 log reduction. Survival of pathogens after a 1kGy e-beam treatment was not affected by the presence of meat surface fat or by storage at 4 °C.

Irradiation at doses of ≤ 1 kGy did not affect the overall ratings of beef aroma, off-aroma, tenderness, juiciness, beef flavour and off-flavour of treated ground beef patties even when made with 100% irradiated beef.

Raw, irradiated intact beef muscles were more brown compared to non-irradiated muscles, but displayed less off-aroma after 14 d storage at 4 °C. This difference in colour is unlikely to affect consumer acceptance, as irradiation would only affect the surface of muscles and once ground, the irradiated portion would represent a fraction of the total

blended product and any colour difference would be imperceptible after cooking.

Overall, 1 kGy e-beam treatment was able to inactivate *E. coli* O157:H7, non-O157 VTEC, as well as *Salmonella*, but the latter to a lesser extent. With no major sensory changes, this treatment has the potential to be an effective antimicrobial intervention in the beef processing industry.

CHAPTER 7

7. Recommendations for future research

Since the D values in the cell-suspended buffer tests were based on a dose of up to 300 Gy due to the limited capacity of the machine, it would be valuable to obtain D values at a dose of ≤1 kGy in meat at controlled temperatures, since these organisms may be more radiation resistant when treated in meat.

Salmonella, being more resistant, may require the combination of more than one intervention method to cause its complete inactivation. Expanding the irradiation study to include a lactic acid treatment may be beneficial.

Although enhanced red-colour of irradiated beef was not observed in this study, it seems to be a common result observed in other studies. Further studies to better understand the stability of vacuum-packaged irradiated beef colour is needed.

Further work could also focus on understanding the adaptability of these pathogens to irradiation. Evaluation of the risk of developing radiation-resistant strains would be of value.

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