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Sensitivity and survival of Escherichia coli O157:H7 isolates after X-ray and electron beam irradiation in raw ground meat

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Sensitivity and survival of *Escherichia coli* O157:H7 isolates after X-ray and electron beam irradiation in raw ground meat

by

Adelle Denise Chan

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Co-majors: Meat Science; Food Science and Technology
Major Professors: Dennis G. Olson and James S. Dickson

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This is to certify that the Master's thesis of

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has met the thesis requirements of Iowa State University

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TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION

Introduction

The usefulness of irradiation as a means of food preservation has been recognized for many years. However, its application in the food industry has been delayed due to regulatory aspects, equipment availability, and the unfamiliarity of consumers with this process. More importantly, there has been concern over the safety and wholesomeness of consuming irradiated foodstuffs. However, it has been established from over 40 years of extensive research, that at doses permitted for commercial food applications, irradiated foods are not hazardous to human health (Diehl, 1995). In the United States, the irradiation of poultry and pork is permitted for pathogen and parasite control, respectively (Engel *et al.*, 1988; Pszczola, 1993). Currently, the Food and Drug Administration [FDA] is reviewing a petition to allow the irradiation of fresh and frozen raw red meat for the elimination of pathogens (FDA, 1994).

Since 1982, *Escherichia coli* O157:H7 has been recognized as a foodborne pathogen. Although this organism has been mainly associated with the consumption of undercooked ground meat, sporadic outbreaks have linked *E. coli* O157:H7 with a variety of vehicles. Non-bloody diarrhea, bloody diarrhea (hemorrhagic colitis), hemolytic uremic syndrome, and thrombotic thrombocytopenia purpura are clinical manifestations of *E. coli* O157:H7 poisoning. Those individuals especially susceptible to infection include the young (<5 yr. old) and the elderly (Padhye and Doyle, 1992; Neill, 1994).

E. coli O157:H7 can be distinguished from other *E. coli* because it does not ferment sorbitol, lacks the enzyme β-glucuronidase, and grows poorly at 44-45°C (Griffin and Tauxe,

1991; Doyle and Schoeni, 1984). Much research has been conducted on studying the prevalence of the organism, microbiological characteristics, and clinical syndromes. Moreover, new detection methods and decontamination procedures have been examined, including food irradiation (Padhye and Doyle, 1992; Thayer and Boyd, 1993).

Research conducted by the National Animal Disease Center in Ames, Iowa demonstrated that 25 *E. coli* O157:H7 isolates, obtained from a national prevalence study in dairy cattle, differed from each other in their resistance to antibiotics (Cray, Jr., 1994). Therefore, it was the purpose of this study to determine whether these strains differed in their resistance to irradiation and to examine the irradiation processing parameters of the petition.

Thesis Organization

This thesis is organized as a literature review (chapter 2), one paper (chapter 3) and general conclusions (chapter 4). The paper entitled "Sensitivity and survival of *Escherichia coli* O157:H7 isolates after x-ray and electron beam irradiation in raw ground beef" was co-authored with Dr. James S. Dickson, Dr. Dennis G. Olson, Dr. William C. Cray, Jr., Dr. E.A. Murano, and Dr. Alicia Carriquiry and will be presented at the Institute of Food Technology's 57th Annual Meeting held in Orlando, Florida on June 14-18, 1997. The paper will also be submitted for publication to the Journal of Food Protection. Finally, reference citations throughout the thesis were formatted following the Journal of Food Protection Instructions for Authors.

References

Cray, Jr., W.C. 1994. (Unpublished data).

Diehl, D.F. 1995. Safety of irradiated foods. Marcel Dekker, Inc., New York, NY.

Doyle, M.P., and J.L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48:855-856.

Engel, R.E., A.R. Post, and R.C. Post. 1988. Implementation of irradiation of pork for Trinchina control. Food Technol. 42:71-75.

FDA. 1994. Filing of food additive petition. Notice. U.S. Food and Drug Administration. Fed. Regist. 59:43848-43849.

Griffin, P.M., and R.V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60-98.

Neill, M.A. 1994. *E. coli* O157:H7 time capsule: what do we know and when did we know it. Dairy Food Environ. Sanit. 14:374-377.

Padhye, N.V., and M.P. Doyle. 1992. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. J. Food Prot. 55:555-565.

Pszczola, D. 1993. Irradiated poultry makes U.S. debut in Midwest and Florida markets. Food Technol. 46:89-92,94,96.

Thayer, D.W., and G. Boyd. 1993. Elimination of *Escherichia coli* O157:H7 in meats by gamma irradiation. Appl. Environ. Microbiol. 59:1030-1034.

CHAPTER 2. LITERATURE REVIEW

Food Irradiation

History of development

Although food irradiation is considered a "new" technology, the idea of exposing bacteria to ionizing radiation was first explored over a century ago. Biological effects were examined by Minck soon after the discovery of X-rays in 1895 by Wilhelm Röetgen and radioactivity in 1896 by Henri Becquerel (Goldblith, 1966).

With the discovery of nuclear fission and the development of accelerators, numerous feasibility studies were conducted worldwide. The first significant experiments demonstrating the preservation capacity of ionizing energy were carried out in the 1940's. Hamburger meat had been preserved by X-rays and independent from this, the first scientific paper on radiation preservation was published (Goldblith, 1966; Diehl, 1995).

By the early 1950's, national food irradiation research programs were established in many countries. The National Food Irradiation Program, in the U.S., came about as a result of President Dwight D. Eisenhower's "Atoms for Peace" program. The U.S. Atomic Energy Commission and the U.S. Army Quartermasters Corps conducted experiments evaluating the wholesomeness of foods treated with low and high doses of irradiation. In 1965, it was concluded that foods irradiated up to 56 kGy were safe for human consumption (Diehl, 1995; Dempster, 1985; Olson, 1995).

In 1969, the Joint Expert Committee (JEC) was formed to evaluate the wholesomeness of irradiated foods. The committee included scientists from the Food and

Agriculture Organization [FAO], the International Atomic Energy Agency [IAEA], and the World Health Organization [WHO]. In 1980, after reviewing all available data, including the Barna review of 1223 studies on the wholesomeness of 278 foods, it was concluded that all doses up to 10 kGy presented no toxicological hazards and introduced no special nutritional or microbiological problems. It was also recommended that toxicological testing of foods irradiated up to 10 kGy was no longer required (Barna, 1979; Sahasrabudhe *et al.*, 1989).

Status of irradiation

In the 1960's wheat, wheat powder, and white potatoes received the first approval for irradiation by the FDA. Since then approval has been given to spices and seasonings, pork, fresh fruits, poultry, and dry or dehydrated enzyme preparations and vegetables (ADA, 1996).

The U.S. has 40 licensed irradiation facilities of which 16 irradiate spices for wholesale use (ADA, 1996). In 1992, the first commercial food irradiator in the U.S. was commissioned for operation. Food Technology Services (previously Vindicator Inc.), a gamma ray facility, is located in Mulberry, Florida (Marcotte, 1992). In the following year, the country's first pilot food irradiator, an electron beam/X-ray facility, started operating at Iowa State University in Ames, Iowa.

All irradiated foods in the U.S. must bear the international symbol for irradiation, the "Radura", and the words "treated by irradiation" or "treated with radiation". Products that contain irradiated ingredients such as spices are not required to be labelled (ADA, 1996).

Worldwide, 36 countries permit the irradiation of food of which 26 irradiate food commercially including Brazil, France, the Netherlands, Israel, South Africa, and Japan. A

variety of products are available; however, clearances differ from country to country. (Loaharanu, 1994, 1989).

Consumer acceptance

In a survey conducted by Resurreccion *et al.*(1995), 446 participants responded to a questionnaire designed to determine current consumer attitudes towards irradiation. It was concluded that the overall attitude towards irradiation was favorable and that awareness among the public had increased. More importantly, results indicated that a more positive response would be observed once irradiated poultry, red meat, and seafood became more available; 69 - 82% of the respondents believed that there was some degree of necessity for the irradiation treatment of these food items. As for global acceptance, it was found that the understanding of this technology is extremely limited. In spite of this obstacle, once the public was presented with scientifically-based information demonstrating the benefits of the irradiation process, more consumers were willing to purchase irradiated products (Bruhn, 1995).

Market response to irradiated mangoes, papayas, strawberries, and chicken have been well received in the U.S. (Bruhn and Schutz; 1989; Marcotte, 1992; Pszczola, 1993). In addition, reports show that a significant proportion of consumers are willing to pay a higher price than what they currently paying for non-irradiated product (Hashim *et al.*, 1994).

Technical Aspects of Food Irradiation

Food irradiation is a process that involves the use of ionizing radiation from gamma (γ) rays and machine sources. Briefly, the commodity to be treated is exposed to the radiation field for a controlled period of time, energy is absorbed by the food, and it is this energy that imparts the observed desired or undesired effect.

Radiation

The term radiation refers to energy that is either emitted as electromagnetic waves or as an energetic nuclear particle (corpuscular radiation). Electromagnetic radiation involves the transmission of energy bundles, called photons, through space or matter by means of oscillating electric and magnetic fields. Examples of electromagnetic radiation include: radio waves, microwaves, infrared heat waves, visible light, ultraviolet rays, X-rays and γ rays. Corpuscular radiation, on the other hand, involves the motion of particles which possess mass and kinetic energy. Examples of corpuscular radiation include: electrons, protons, neutrons, and alpha (α) particles (Urbain, 1986; CAST, 1986).

Ionizing radiation

Ionizing radiation refers to those photons and particles which possess energy at high enough levels to cause the ejection of orbital electrons from an atom. These newly released electrons can then sequentially excite or ionize other molecules. In contrast to ionized molecules, the electrons of excited molecules are only displaced to another orbital of higher energy. Those radiations that are capable of inducing ionization have several million electron volts (MeV) of

energy. In food irradiation, γ-rays and machine generated X-rays and electrons are employed (CAST, 1986; Urbain, 1986).

Irradiation dose and dose rate

The SI name for the amount of energy absorbed by a substance (i.e. food) is the gray (Gy); 1 gray is equal to 1 Joule of energy absorbed by 1 kg of matter. For food irradiation purposes, the dose is often expressed in kilograys (kGy). The former unit of measure was the rad; one rad is defined as 100 ergs/g. The conversion from rads to grays is simple, 100 rad = 1 Gy, or 1 Krad = 10 Gy, or 1 Mrad = 10 kGy. The dose applied will vary with the commodity being irradiated and the dose measured depends on the length of time the substance is exposed to the radiation field. It is also important to note that irradiation is considered a "cold" process because the quantity of heat generated is negligible; 10 kGy is the amount of energy needed to raise the temperature of water by 2.4°C (Diehl, 1995; Satin, 1993).

The rate at which energy is transferred to the substance per unit time is called the dose rate. Dose rate differs with the irradiation source. Gamma ray sources deliver a relatively low dose rate (0.1 -10 kGy/h), while electron accelerators deliver a high dose rate (10 kGy - 10^6 kGy/h). Irradiation with gamma rays, therefore, will require a longer processing time to deliver the final desired dose (Diehl, 1995).

Sources of Irradiation for the Treatment of Food

To be of any practical use, the type of radiation used must be capable of penetrating food products, does not induce radioactivity, and can be generated in sufficient quantities at an acceptable cost.

Electrons

Of the particles that produce corpuscular radiation, only accelerated electrons are suitable for the treatment of food. Electron beam accelerators can generate high-speed high-energy electrons in sufficient quantities for food applications. Due to the electric charge of this particle, electrons can be focused magnetically to form an electron beam, which in turn, can be directed at the target (absorber). The effective penetration range of an electron beam depends on its energy level and the density of the product. Electrons of energies at least 5 MeV can penetrate a thickness of 1.9 cm (0.75 in.) and at 10 MeV, the thickness is 4.5 cm (1.75 in.) in a medium with a density of $1g/cm^3$. The penetrability is improved when both sides of the product are treated (CAST, 1986; Urbain, 1986; Olson, 1995).

Electron accelerators

Two basic designs of high-energy electron accelerators are available: the direct current (DC) accelerator and the indirect or radiofrequency (RF) linear accelerator (linac). In both systems, electrons are accelerated in a vacuum tube to almost the speed of light. This is accomplished by applying a high voltage across the terminals of the vacuum tube. Electrons emitted from a heated filament or an "electron gun" are directed towards the positive end of

the tube where a scanning magnet then directs the electron beam to the target below. By moving the material perpendicular to the scanning line of the electron beam, an even distribution of radiation energy to the target material can be achieved (Diehl, 1995).

The DC accelerator is limited to energy levels no higher than 5 MeV, whereas linacs can produce energies above 10 MeV and are more suited for food irradiation applications. Because of their relatively low depth of penetration electron beams cannot be used for the irradiation of animal carcasses, large packages, or other thick materials. Nevertheless, this is overcome by converting electrons to X-rays (Diehl, 1995).

X-rays

Electron kinetic energy can be converted to X-rays to improve penetration. This is achieved when a metal target (i.e. tungsten) is placed in the path of high-energy electrons. Because the overall conversion is very low, less than 10%, X-rays are not economical for commercial scale food irradiation. A maximum energy of 5 MeV is permitted. The advantage of machine-generated radiation is that the power can be turned off when not in use and disposal of waste is not a concern (Olson, 1995).

Gamma rays

Gamma rays are obtained from the decay of Cesium-137 (Cs-137) or Cobalt-60 (Co-60). Cs-137 is a nuclear waste by-product that can be extracted from spent fuel rods in the form of CsCl, a water soluble compound. Not many facilities that reprocess spent nuclear fuel exist; thus, quantities of Cs-137 are limited and rarely used in gamma radiation facilities. Co-

60, on the other hand, is obtained by activating Co-59 with neutrons in a nuclear reactor. It is available in water insoluble form. Co-60 disintegrates to stable Ni-60 with the emission of gamma radiation at energy levels of 1.17 and 1.33 MeV. Both Cs and Co are doubly or triply encapsulated in stainless steel and lowered into a storage pool when not in use. Gamma irradiators are the dominant power source for irradiators worldwide. Finally, X-rays (5 MeV) and γ rays have a penetration range of about 30 cm (Diehl, 1995; CAST, 1986).

The Effects of Ionizing Radiation

As mentioned previously, ionizing radiation has the ability to displace or eject electrons of absorber atoms by energy transfer. An excited molecule will undergo de-excitation by giving off energy in the form of light or heat, by transferring energy to another molecule, or by chemical reactions. These chemical reactions can result in the production of either stable molecules or very unstable free radicals. Moreover, an ion will either react with another ion of the opposite charge or undergo reactions similar to excited molecules to also produce free radicals. These short-lived primary reactive species initiate chemical reactions that will affect the structure of macro- and micromolecules giving rise to the biochemical, physiological, and biological effects observed in irradiated foods (Urbain, 1986; Diehl, 1995).

In food systems, reactive species derived from water are mainly responsible for the effects of irradiation. When pure water is irradiated, a number of radiolytic products are formed: hydrogen atoms ($\bullet H$), hydroxyl radicals ($\bullet OH$), hydrated electrons (e^-_{aq}), hydrogen peroxide (H_2O_2), and hydrated protons (H_3O^+).

The overall equation for the irradiation of water is:

$$H_2O \xrightarrow{\text{radiation}} {\cdot}H + {\cdot}OH + e^-_{aq} + H_2 + H_2O_2 + H_3O^+$$
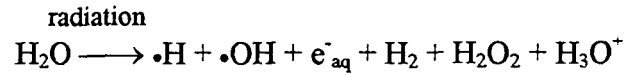
These reactive species can combine to form stable molecules or further cause the excitation or ionization of other atoms (e.g. molecular oxygen, other water reaction products, and food components). In frozen foods, however, the reactive species are immobilized and effects arise mainly from the direct interaction of photons and electrons with food components (i.e. nutrients) and contaminants (i.e. bacteria). This is also the case with dehydrated products (Urbain, 1986; Diehl, 1995).

The presence or absence of oxygen during irradiation can also have a profound effect on the food product, especially those containing lipids. In addition to reacting with water breakdown products, oxygen can form peroxide radicals which contribute to rancidity and off-flavors (Urbain, 1986; Diehl, 1995).

Effects of ionizing radiation on nutrients

Through nutrient analyses, animal feeding tests, and human studies, it has been established that the irradiation of foods at legal doses does not adversely affect product wholesomeness (WHO, 1988).

Carbohydrates

The main effect of irradiation on carbohydrates (CHO) is the breaking of the glycosidic bond in polysaccharides. Polysaccharides are broken down to smaller units which

in turn, can affect product quality and functionality. Starches and cellulose are made more susceptible to enzyme hydrolysis (i.e. reduction in viscosity and softening of fruits and vegetables, respectively) while pectins lose their gelling properties (Murano, P., 1995; Diehl, 1995; CAST, 1986).

Proteins

Irradiation can denature proteins by breaking hydrogen bonds and other linkages involved in secondary and tertiary structures, thus causing the unfolding of the protein. This may result in tenderizing of meat products. The doses that are employed for food irradiation normally do not affect enzyme activity or protein availability and digestibility. Furthermore, it has been noted that proteins and amino acids can act as protectants for carbohydrates from degradation (Murano, P., 1995; Urbain, 1986; Diehl, 1995; CAST, 1986).

Lipids

Irradiation can promote the development of rancidity of unsaturated fats. However, removal of oxygen, protection from light, the presence of antioxidants, and freezing of the product can minimize this problem during processing (Murano, P., 1995; Urbain, 1986; CAST, 1986).

Vitamins

The water-soluble vitamins thiamin and ascorbic acid are the most sensitive to irradiation while pantothenic acid, folate, and B12 are the most resistant. In the case of

vitamin C, irradiation converts it into dehydroascorbic acid (DHA) which possesses activity similar to its parent compound. Of the fat-soluble class, vitamins A and E are affected to some degree. As with lipids, by excluding oxygen and light and freezing the product, damage is reduced. In addition, vitamin C can act as antioxidant, and thereby protect other vitamins from degradation (Murano, P., 1995; Urbain, 1986).

Effects of ionizing radiation on microorganisms

The elimination of spoilage and pathogenic bacteria is the main objective in treating food with ionizing radiation. The decontamination of foods is achieved through the direct or indirect interaction of radiation with bacterial DNA. Indirect effects are the result of the reactive water species formed in the cell. On the other hand, in a bacterial spore, nucleic acids directly interact with radiation. Hence by damaging the structure of DNA, cell injury (i.e. loss of prolific and metabolic function) or death occurs. Organisms differ in their sensitivity to radiation, listed in decreasing order of resistance are: viruses, bacterial spores, vegetative bacterial cells, yeasts and molds, and parasites (Diehl, 1995; Murano, E., 1995).

Survival curves

Microbial populations decrease exponentially with increasing irradiation dose and the sensitivity of an organism to radiation is expressed as a D_{10} value, the dose required to reduce the population by one log or 90%. To determine irradiation D_{10} values, survival or dose-response curves are constructed (Figure 1). The log surviving fraction is plotted as a function of dose. This relationship is mathematically expressed as:
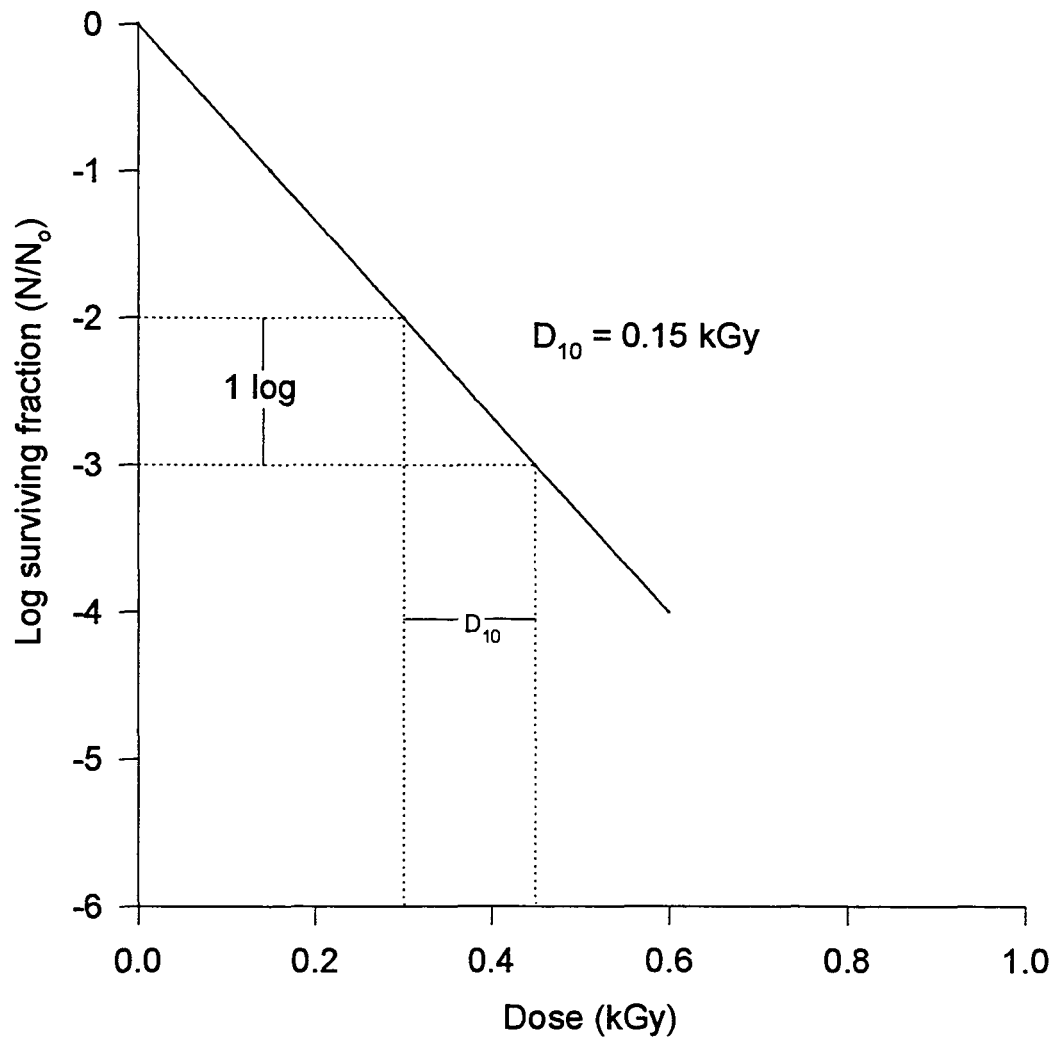
Figure 1. A typical dose-response curve.

$$\log_{10} \frac{N}{N_o} = - \frac{D}{D_{10}}$$

where N_o is the initial number of organisms, N is the number of organisms surviving after a dose D. N/N_o is referred to as the surviving fraction and the slope of the curve is $-1/D_{10}$.

In some incidences, as seen with radiation-resistant microorganisms, a shoulder can be observed on a survival curve before the linear slope begins. In that region, low doses have been delivered and little or no inactivation occurs because the organism is able to repair damaged DNA. However, when a higher dose is utilized, damage is too great and cell death starts proceeding exponentially. Consequently, if the D_{10} value is calculated from the exponential portion of the curve, this will result in an underestimation of the "true" resistance of the organism. To take the shoulder effect into consideration another mathematical model is used (Diehl, 1995; Tarté, 1996).

Factors which influence the survival of microorganisms

The ability of microorganisms to survive irradiation is influenced by several factors: genus and species type, temperature, composition of medium, dose rate, atmosphere, post-irradiation incubation and combination treatments. Below, these factors are briefly discussed.

Sensitivity of a microbe to irradiation depends on its ability to repair DNA damage. *Deinococcus radurans* (previously *Micrococcus*) was found to be the most gamma radiation-resistant of 36 vegetative microorganisms. It could survive at -80°C in phosphate buffer at 24 kGy, whereas the majority other the other bacteria could not survive at 3 kGy. Furthermore, its survival curve exhibited a shoulder (Anellis *et al.*, 1973).

As temperature decreases, the D_{10} value of an organism increases. The protective effect of decreasing temperature is explained by the reduction in migration of free radicals and excited molecules throughout the product (Urbain, 1986).

The composition of the target material plays a role in radiation sensitivity. Patterson (1989) noted that *Listeria monocytogenes* was more resistant in poultry meat than in phosphate-buffered saline.

At high dose rates, oxygen can be depleted at a rate greater than it can be replaced by atmosphere diffusion into the food. This can create an anoxic environment and therefore, increase the resistance of organisms (Urbain, 1986). It has also been postulated that recombination of radicals is favored over the reaction of radicals with food components. Studies by Fu *et al.* (1995) and Tarté *et al.* (1996), however have shown that there is no affect on the survival of pathogens.

The atmosphere in which the product is irradiated can affect the recovery of organisms. As indicated in several studies cited by Tarté *et al.*(1996), depending on the organism, the D_{10} values may or may not change when irradiated under air, vacuum, CO_2, and N_2.

When calculating the D_{10} value of an organism, the composition of the recovery media must be considered. Numerous studies have evaluated media for the detection of irradiation survivors, recovery of organisms on non-selective media is greater than on selective media (Blank and Corrigan, 1995; Tarté R. *et al.*, 1996).

Finally, the combination of irradiation and other preservation methods can have synergistic effects. Low dose gamma irradiation (0.8 kGy) sensitized *Listeria monocytogenes* to heat treatment (Grant and Patterson, 1995). Furthermore, Crawford *et al.* (1996)

demonstrated that treatment of *Clostridium sporogenes* spores in chicken meat with high

hydrostatic pressure prior to irradiation resulted in a lower D_{10} value, thereby reducing the

amount of radiation needed to extend the shelf-life of poultry.

Food Irradiation Applications

Depending on the dose, the microbial population can be reduced or completely eliminated

to achieve short term or long term extension of the shelf-life of foods. There are three major

classifications of radiation treatment (Table 1). Radurization or radiation pasteurization involves

doses up to 3 kGy for the purpose of reducing the number of spoilage microorganisms in a food.

The elimination of non-spore forming pathogenic bacteria and the inactivation of parasites can be

achieved by using the doses employed in radicidation, 3 to 10 kGy. Both radurization and

radicidation can extend the shelf-life of products from days to weeks. Radappertization is the

radiation-sterilization of all microorganisms with radiation doses of 10 to 50 kGy. As a result,

refrigeration of these food products is not necessary; the product is stabilized for months or years.

Table 1. Applications of Food Irradiation[a]

Product	Purpose	Dose
Potatoes and onions/ cereals and pulses/ fruits and vegetables etc.	Sprout inhibition/ insect and parasite disinfestation/ delay of ripening	0.05-1.0 kGy
Fresh fish, strawberries, etc.	**Radurization:** Shelf-life extension	1-3 kGy
Fresh and frozen seafood, meat and poultry	**Radicidation:** Elimination of spoilage and pathogenic microorganisms	3-10 kGy
Medical equipment, dry food ingredients	**Radappertization:** Sterilization	10-50 kGy

[a]Modified from Sahasrabudhe *et al*. (1989) and WHO (1988).

Irradiation preservation of meats

The emergence of "new" pathogenic bacteria has given rise to increased interest in using irradiation as a preservation technique in the food industry. In the U.S. and Canada, most foodborne outbreaks are linked to the consumption of meat, poultry, and related products; therefore, irradiation provides a means to prevent unnecessary illness and death (Bean and Griffin, 1990; Todd, 1988).

Irradiation of pork at a dose of 1.0 kGy has been permitted by the FDA since 1985 for the purpose of controlling *Trichinella spiralis*. This dose is also sufficient to eliminate *Toxoplasma gondii* and the larvae of the beef and pork tape worms *Cysticercus bovis* and *C. cellulosae*, respectively (Clavero *et al.*, 1994; Thayer, 1993). Three kilograys has been approved for the elimination of salmonellae in poultry, whereas research has demonstrated that irradiation is also effective in controlling *Campylobacter jejuni* and *Listeria monocytogenes* (Clavero *et al.*, 1994; Pszczola, 1993). Currently there is a petition to the FDA to irradiate fresh and frozen red meats at 1.5- 4.5 kGy and 2.5 - 7.5 kGy, respectively, for purposes of controlling pathogens such as *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, etc. (FDA, 1994).

Other effects on meats

Irradiation of meat used for the production of fermented sausage allows for a lower inoculum of lactic acid bacteria as the total aerobic bacteria, coliform and staphylococci counts are reduced (Dickson and Maxcy, 1985).

The amount of nitrite added to cured hams can be decreased. Frozen hams (25 mg/kg $NaNO_2$) were subjected to 37 to 44 kGy and were found not to be objectionable in color, odor, flavor, and overall appeal when compared to non-irradiated hams (156 mg/kg $NaNO_2$). In addition, *Clostridium botulium* toxin was not a threat (Dempster, 1985).

With respect to sensory properties, numerous studies have revealed that under optimum conditions (i.e. dose, temperature, and atmosphere), irradiated foods have compared favorably to non-irradiated controls. For sterilization purposes, it has been determined that vacuum packaged frozen products are the most desirable in terms of sensory characteristics (Dempster, 1985). According to a study cited by Thayer (1993), Urbain and Giddings reported that vacuum packed beefsteaks treated with phosphate retained better color and received sensory panel ratings comparable to control samples.

The presence of antioxidants can improve the quality of foods containing lipids. Ascorbic acid and α-tocopherol contributed to the reduction of off-flavor in chicken (Patterson and Stevenson, 1995). It has also been shown that irradiation, in conjunction with modified packaging, has a favorable effect on minced pork safety and sensory properties (Grant and Patterson, 1991).

Detection of Irradiated Foods

Distinguishing irradiated foods from non-irradiated foods is a concern of consumers and regulatory officials. Therefore, methods to detect physical, chemical, and biological differences have been developed. A few methods that relate to meat and poultry products are briefly described here.

O-tyrosine is produced when hydroxyl radicals react with the amino acid phenylalanine; this product has been identified and quantified by high performance liquid chromatography. A study by Chuaqui-Offermanns and McDougall (1991) revealed a linear relationship between irradiation dose and the yield of *o*-tyrosine.

Electron spin resonance (ESR) has been used to detect free radicals that are "trapped" in the matrix of bones and seeds (Desrosiers, 1991; Dodd *et al*., 1992).

A microbiological method has been described. *Aeromonas hydrophila* has been found to produce 40-50% less volatile acids (TVA) and volatile bases (TVBN) on fresh and frozen irradiated meat, poultry and fish. The authors established values that could be used to distinguish non-irradiated and irradiated flesh products (Alur *et al*., 1992).

Boyd *et al*. (1991) discovered a compound that was unique to irradiated chicken meat which could serve as a marker in other foods that contain lipids. 2-Dodecylcyclobutanone was not found in non-irradiated raw or cooked chicken meat.

Escherichia coli

General characteristics

E. coli is a gram-negative, facultative, non-spore-forming bacillus found in the environment as well as the intestines of animals and humans. Originally named *Bacterium coli commune*, it was first isolated by Esherich in 1885 (Jay, 1992). *Escherichia* represents one of four genera that constitute the fecal coliform bacteria that are defined by lactose fermentation within 48 hours, the production of gas and acid in EC broth between 44 and 46°C, and appearance of dark colonies with a metallic sheen on Endo-type Agar. *E. coli* type I strains,

which are targeted for coliform testing, produce indole, are methyl red positive, Voges-Proskauer negative for acetoin production, and do not utilize citrate. Type II strains, in contrast, are only methyl red positive. The pH growth range is 4.3 - 9.0 and minimum a_w is 0.96 (Jay, 1992).

E. coli O157:H7, specifically, grows rapidly between 30-42°C with generation times ranging from 0.49 h at 37°C to 0.64 h at 42°C. The organism grows poorly at 44-45°C and does not grow within 48 h at 10 or 45.5°C. The temperature range for *E. coli* O157:H7 growth and gas production in *E. coli* medium within 48 h is 19.3 to 43.0°C (Doyle and Schoeni, 1984; Raghubeer and Matches, 1990). Therefore, normal screening methods for fecal coliforms would fail to detect *E. coli* O157:H7. This serotype is very acid-tolerant (Miller and Kaspar, 1994). The organism survives at pH 2 and it can grow in tryptic soy broth at concentrations up to 6.5% NaCl (Glass *et al.*, 1992).

Most biochemical reactions of *E. coli* O157:H7 are typical of *E. coli* with the exception of sorbitol fermentation and β-glucuronidase activity. *E. coli* O157:H7 does not ferment sorbitol within 24 h (Wells *et al.*, 1983). Furthermore, the organism lacks the enzyme β-D-glucuronidase, and therefore cannot hydrolyze the indicators 4-methyl-umbelliferone glucuronide (MUG) or 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (BCIG) (Okrend *et al.*, 1990a,b).

Serotyping

Kauffman established the serotyping of *E. coli* in the late 1940's based on different antigens (Bettelheim, 1996). The four major types of surface antigens which are used in the serological classification of *E. coli* are: the O (somatic), H (flagellar), K (capsular), and F (fimbriae) antigens. The O and K antigens are polysaccharides and the H and F antigens are proteins. About 171, 56, and 100 of 0, H, and K, respectively, are known. However, only about 30 serovars are associated with human diarrheal disease (Jay, 1992; Molenda, 1994).

Disorders Caused by *Escherichia coli*

Gastrointestinal illness caused by *E. coli*

E. coli strains implicated in foodborne gastroenteritis may be classified into five categories: enteropathogenic, enterotoxigenic, enteroinvasive, enteroadhesive, and enterohemorrhagic. In addition to serotypes, these *E. coli* groups are distinguished by distinct virulence properties, different interactions with intestinal mucosa, distinct clinical syndromes, and differences in epidemiology.

Enteropathogenic *E. coli* (EPEC) is an important cause of infant diarrhea. These organisms possess the EAF factor, a 60 MDa plasmid, that encodes the property of adhesiveness to HEp-2 (human larynx) cells (Kornacki and Marth, 1982; Levine, 1987). Enterotoxigenic *E. coli* (ETEC) has been commonly associated with travellers' and infant diarrhea in developing countries. These strains produce either a heat-stabile (ST) or heat-labile (LT) enterotoxin or both. ETEC attach to epithelial cells by means of fimbriae. Illness is similar to cholera, but much milder (Reed, 1994; Levine, 1987). Enteroinvasive *E. coli*

(EIEC) causes bloody diarrhea and a syndrome resembling shigellosis. Enteroadhesive *E. coli* (EAEC) causes infantile diarrhea. EAEC are distinguished from the aforementioned groups in that they do not produce LT, ST, or Shiga-like toxin, are non-invasive, and do not possess EAF. Furthermore, they adhere to HEp-2 cells in a characteristic manner (Levine, 1987; Molenda, 1994).

The enterohemorrhagic *E. coli* group (EHEC), of which *E. coli* O157:H7 belongs, is responsible for non-bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. Furthermore, the production of verotoxins (VT) or shiga-like toxins (SLT) have been associated with these organisms. In addition to diarrheal disease, pathogenic *E. coli* is also responsible for extra-intestinal infections including: urinary tract infections, neonatal meningitis, wound infections, peritonitis, and septicemia (Molenda, 1994).

Hemorrhagic colitis

Hemorrhagic colitis (HC) is characterized by a sudden onset of severe abdominal cramps, followed by the development of watery and later grossly bloody diarrhea. Vomiting may occur, but there is little or no fever. The incubation period ranges from 3 to 9 days and the duration of illness lasts from 2 to 9 days. A barium enema reveals a tumour-like appearance in the ascending and traverse colon in patients and antibiotic use does not aid in recovery (Riley *et al.*, 1983). Patients with non-bloody diarrhea typically do not seek medical care, this leads to an under-reporting of cases (Griffin and Tauxe, 1991). Potential risk factors for developing HC include age, infectious dose, prior or concurrent antimicrobial use,

and decreased stomach acidity (Griffin and Tauxe, 1992; Tarr *et al.*, 1988; Neill, 1989). The highest attack rates have been reported in children under 5 years of age and the elderly with a range of ages from 11 months to 78 years. A proportion (5-10%) of HC cases progress to hemolytic uremic syndrome (Padhye and Doyle, 1992; CDC, 1994).

Hemolytic Uremic Syndrome

Hemolytic Uremic Syndrome HUS) was first described in the mid-1950's by Van Gosser *et al.* (Bettelheim, 1996). HUS is the leading cause of acute renal kidney failure in children. Dialysis and blood transfusions are often necessary. Specifically, the endothelial cells are damaged in HUS patients. This leads to the clotting of the kidney capillaries and the subsequent accumulation of waste products. The case fatality rate is 3-5% (CDC, 1994). Studies reviewed by Padhye and Doyle (1992) have shown that the incidence rates have increased from the 1970's to the 80's. It has also been noted that females are at a greater risk for the progression of HC to HUS.

Thrombotic Thrombocytopenic Purpura

Thrombotic Thrombocytopenic Purpura (TTP) is similar to HUS except the central nervous system is involved. It is a syndrome usually occurring in adults that consists of microangiopathic hemolytic anemia, thrombocytopenia, renal failure, fluctuating neurological signs, fever, and mild azotemia. Patients often develop blood clots in the brain and death frequently follows. Risk factors for developing HUS and TTP include: age, gender, mental

retardation, bloody diarrhea, toxin type of infecting strain, and antimicrobial therapy (Griffin and Tauxe, 1991; Ostroff *et al.*, 1989).

Other unusual clinical complications associated with *E. coli* O157:H7 infection include hemorrhagic cystitis and balanitis, convulsions, sepsis with another organism, and anemia (Padhye and Doyle, 1992).

Pathogenicity

Virulence factors that may contribute to the pathogenicity of *E. coli* O157:H7 include: the production of one or more verotoxins, the production of an enterohemolysin, the 60 MDa plasmid, colicin D production, the presence of the *eae* gene which is said to be involved in the adherence and effacement of microvilli, and surface structures other than pili or fimbriae for adhesion to intestinal cells.

Verotoxins

Verotoxins (VT) were first described by Konowalchuk in 1977. Culture filtrates from some *E. coli* strains were found to be cytotoxic to vero cells obtained from African green monkey kidneys. To date, several verotoxins have been identified: VT 1, VT 2, human variants of VT 2, and animal variants.

VT 1 behaves similarly to *Shigella dysentariae* I (Shiga) toxin. It was cytotoxic to HeLa (human cervix) cells, lethal to mice, enterotoxic to rabbits, and neutralized by Shiga antiserum, and thus termed Shiga-like toxin (SLT 1) (O'Brien *et al.*, 1983). VT 2 or SLT 2 is

not neutralized by Shiga toxin antisera and is found predominantly in culture filtrates, whereas VT 1 is found in cell lysates (Padhye and Doyle, 1992).

Both VT 1 and VT 2 consist of subunits A and B. Subunit B is further comprised of 5 smaller units. The MW of VT 1 subunits A and B are 33,125 and 7,187, respectively. As for VT 2, the MW are 35,000 and 10,700 for A and B, respectively (Padhye and Doyle, 1992). Furthermore, the genes mediating VT 1 and 2 expression are bacteriophage encoded (Scotland *et al.*, 1985) and the structural genes for VT 1 and 2 share 58% overall nucleotide sequence homology (Jinneman *et al.*, 1995). In addition, the gene for VT in one of the human VT 2 variants is located in the bacterial chromosome (Lior, 1994).

The receptor for VT 1 and 2 is a globotrisyl ceramide containing a galactose α-(1-4)-galactose-β-(1-4) glucose ceramide, which is found in the kidney and is present in primary cells of the endothelium. Both VT 1 and 2 possess the same mode of action, the A subunit is responsible for blocking protein synthesis and the B subunit binds the toxin to host cells receptors. Specifically, 60S ribosomal subunits are inactivated as the N-glycosidic bond is cleaved at the adenine-4324 position of 28S ribosomal RNA (Padhye and Doyle, 1992).

E. coli strains from pigs produce a variant of shiga-like toxin II which has been implicated in edema disease of pigs (MacLeod and Gyles, 1989). However, it lacks toxicity for HeLa cells and has a lower LD50 in mice. Beutin *et al.* (1995) found different SLT 2 determinants in goat and sheep, but did not characterize them.

Other virulence factors

Beutin *et al.* (1989) discovered that in addition to VT 1 and VT 2, most verotoxin-producing strains of *E. coli* (VTEC) also produce a unique enterohemolysin (89% of 64 VT positive isolates from human and bovine origin). Furthermore, of 34 *E. coli* O157:H7 human isolates, 32 were enterohemolysin positive. Enterohemolysin is distinguished from α-hemolysin isolates by observing lysis zones on blood agar plates containing washed and unwashed sheep blood.

The 60 MDa plasmid is believed to be responsible for cell adhesion to HEp-2 cells (Levine, 1987) and colicin D has been associated with some *E. coli* O157:H7 strains (Scotland *et al.*, 1987).

Although, the *eae* gene has been associated with the virulence of *E. coli* O157:H7 (Barrett *et al.*, 1992), Louie *et al.* (1994) concluded that the *eae* gene may not be necessary to produce HC or HUS. *Eae* negative non-O157 VTEC were isolated from humans with HC or HUS.

Finally, several studies of Sherman *et al.*, reviewed by Padhye and Doyle (1992), suggest that the adherence of non-piliated and non-fimbriated *E. coli* O157:H7 to Henle 407 (human small intestine) and HEp-2 cells is a function of unidentified outer membrane surface constituents.

Epidemiology

E. coli O157:H7 was first isolated from a patient suffering from grossly bloody diarrhea in 1975 (Griffin and Tauxe, 1991). However, this organism was first recognized as a

foodborne illness-causing pathogen in 1982 in Michigan and Oregon (Riley *et al.*, 1983).
Both outbreaks were linked to the consumption of hamburgers prepared at restaurants
belonging to a single chain. A decade later a larger outbreak of illness due to *E. coli* O157:H7
involving this vehicle and a different fast food restaurant would affect 500 people in
California, Idaho, Nevada, and Washington. In this incident, 4 children died from
complications (Dorn, 1993).

Sporadic cases of *E. coli* O157:H7 poisoning surfaced in North America throughout
the mid-1980's. Residents of nursing homes in Nebraska (1984) and the Canadian provinces
of Alberta (1986) and Ontario (1982) became ill after consuming contaminated ground beef
(Doyle, 1991; Riley *et al.*, 1983). In other outbreaks, ham, turkey, and cheese sandwiches
would be the suspect food items at another Ontario nursing home, while secondary infection
of staff workers by contact was observed (Griffin and Tauxe, 1991). Person-to-person contact
was also responsible in a outbreak reported in the Canadian North West Territories due to
poor sanitation. In 1986, consumption of raw milk proved harmful in Ontario and Wisconsin
dairy farms. A case in Missouri was traced to the municipal water supply in a small town
(Neill, 1994). North Dakotans were afflicted after consuming roast beef and fresh apple cider
was the vehicle in a 1991 Massachusetts outbreak (CDC, 1991; Neill, 1994). Dry-cured
salami was also recognized as a vehicle in 1994 (CDC, 1995). In Oregon, swimming in a
fecally polluted lake would associate the organism with a non-food source (Neill, 1994).

More recently, 4 outbreaks in 1996 in Japan, the Western U.S. and British Columbia,
Connecticut, and Scotland have brought this organism to the spotlight once more. The
suspected vehicles in Japan were fish and raw beef liver. Over 8,300 people, mostly children,

were affected. At least 5 deaths resulted (IASR, 1996). In the West Coast outbreak, 66 cases and one death were linked to the consumption of unpasteurized apple juice. Unpasteurized apple cider was the source of infection in Connecticut. Seven out of 12 people suffered from HUS and 1 from TTP; the ages of those affected were from 2 to 73 years (CDC, 1997 & 1996). Finally, at least 390 cases in Scotland including 10 deaths were suspected or confirmed to be linked to meat supplied by one butcher (Euro Surveillance, 1997).

Infection has also been reported in other European countries, South America, Southeast Asia, and Australasia (Bettelheim, 1996). In two UK outbreaks, yogurt and potatoes were implicated (Morgan *et al.*, 1988, 1993).

Other strains of EHEC

Other serotypes that have been involved in EHEC diarrheal disease include: O26:H11, O26:H21 and O39:H8 in New Zealand; O111:H- and O86:H40 in Italy; O153:H2, O68:H-, O26:H-, and O-:H11 in the U.S.; and, O26:H11, O48:H21, O91:H10, O98:H-, O111:H2, O111:H8, O111:H-, O112a,b:H2, O165:H-, and O157:H- in Australia (CDC, 1995; Bettelheim, 1996).

Sorbitol-fermenting *E. coli* O157

Of 44 strains examined from patients with HUS or diarrhea, 17 O157:H7 strains were found to ferment sorbitol within 24 hours and were positive for β-glucuronidase activity; all 17 were VT 2 positive (Gunzer *et al.*, 1992). Sorbitol-fermenting *E. coli* O157:H- and non-

sorbitol-fermenting *E. coli* O157:H7 strains have a 90-kb plasmid in common, along with
toxin converting phages and the *eae* gene (Karch *et al.*, 1993).

Detection and Isolation

Many approaches have been taken to develop isolation and detection techniques for
this organism, and these can be generally divided into three categories: 1) the use of
biochemical characteristics, 2) the use of DNA probes for virulence markers, and 3)
immunoblotting with antibodies to verotoxins or the O157 and H7 antigens.

Biochemical techniques

The use of sorbitol MacConkey Agar has been a popular choice for the detection of *E. coli* O157:H7. The indicators BCIG and MUG have been added to the formulation of SMAC
to improve its differentiating ability. Non-O157 *E. coli* colonies appear blue when BCIG is
used or fluoresce under UV light in the presence of MUG (Okrend *et al.*, 1990 a, b). Several
investigators have noted that SMAC in general is unsuitable for the recovery of heat and
freeze stressed cells (Abdul-Raouf, 1993; Clavero and Beuchat, 1995). In contrast, phenol
red sorbitol agar containing MUG (PRSA-MUG) is more effective in recovering thermally-
injured cells than SMAC due to its nutrient composition (Ahmed and Conner, 1995).
Incorporation of cefixime and tellurite into SMAC (CT-SMAC) improves *E. coli* O157:H7
recovery as cefixime inhibits the growth of non-sorbitol-fermenting (NSF) *Proteus* spp.,
Morganella morganii, Providencia spp., *Aeromonas* spp., *Plesiomonas* spp., and *Hafnia
alvei*. Moreover, tellurite inhibits non-O157 *E. coli* and other NSF species (Zadik *et al.*,

1993; Bennett *et al.*, 1995; Weagant *et al.*, 1995). Other specific biochemical reactions of *E. coli* O157:H7 can be obtained from Wells *et al.* (1983).

Food Safety and Inspection Service (FSIS)

The FSIS method involves a 6 h enrichment of the sample in modified EC broth containing novobiocin. Serial dilutions are inoculated onto 3M Petrifilm™ *E. coli* count plates. After incubation at 42°C for 18 h, the plates are tested for the presence of the O157 antigen using the Petrifilm™ HEC direct blot ELISA. Presumptive positive spots are then picked off and suspended in 0.85% saline, plated onto SMAC-BCIG, and incubated at 42°C for 18 to 24 h. Typical colonies on SMAC-BCIG are then tested for sorbitol and β-D-glucuronidase reactions on phenol red sorbitol agar (PRS) with MUG. Isolates are also streaked onto eosine methylene blue agar (EMB). Colonies that are sorbitol-negative after 24 h incubation, MUG-negative, and have typical colony morphology and characteristics on EMB are tested for the presence of the O157 antigen by latex agglutination. Isolates possessing the O157 antigen are then tested with H7 antisera. Biochemical confirmational tests include: (+) motility at 35°C, (+) Triple Iron Sugar (TSI) reaction, (-) sorbitol fermentation, (-) cellobiose fermentation, (-) salicin fermentation, (+) indole reaction in tryptone broth, (+) methyl red, (-) Voges-Proskauer reactions, (-) Simmons' citrate reaction, (+) lysine decarboxylase, (+) ornithine decarboxylase, and decarboxylase base (Johnson *et al.*, 1995; Wells *et al.*, 1983).

Probes for virulence markers

Padhye and Doyle (1992) described a gene probe, CV419, developed by Levine and colleagues. It was based on the 60 MDa plasmid and could hybridize with 99% of all *E. coli* O157:H7 and 77% of *E. coli* O26:H11. Furthermore, it hybridized with 81% of other VTEC and only one non-VTEC *E. coli* (99.8% specificity).

The polymerase chain reaction (PCR) for toxin genes has been used in fecal cultures (Cubbon *et al.*, 1996; Gunzer *et al.*, 1992). Deng and Fratamico (1996) developed a multiplex PCR-multiple probe system which could simultaneously amplify sequences of the *eae* gene, the VT 1 and VT 2 genes, and a fragment of the 60 MDa plasmid, and further discriminate between toxigenic O157 EHEC strains and non-toxigenic O157, other serogroups of *E. coli*, and other bacterial species. The detection limit was 65 colony-forming units of *E. coli* O157:H7. Huck *et al.* (1995) attempted to develop a probe that would hybridize with *E. coli* O157:H7 and exclude other VTEC. Of the 38 other genera tested, only *Salmonella bietri* gave a false-positive.

Samadpour *et al.* (1994) developed Shiga-like toxin I and Shiga-like toxin II specific genes probe assays to screen verotoxin-producing *E. coli* in foods and fecal samples. However, Notermans *et al.* (1991) recommended that routine screening of *E. coli* isolated randomly from food for toxin production should be limited to food-borne outbreaks with an etiology resembling an *E. coli* infection. These researchers only detected SLT 1 in 10 of 800 meat and poultry samples.

Immunological techniques

Several immunoassays using antibodies (Ab) to VT 1, VT 2, O157, and/or H7 antigens have been developed. The major disadvantage of immunoassays is that bacteria of different *E. coli* groups and other genera such as *E. hermanii*, *Salmonella* group N species, some *Yersinia enterocolica*, and *Brucella* spp. possess the same somatic antigen residues that are found in *E. coli* (Rice *et al.*, 1992; Chart *et al.*, 1991; Chart *et al.*, 1992; Padhye and Doyle, 1991).

The hydrophobic grid membrane filter (HGMF)-immunoblot procedure developed by Doyle and Schoeni (1987) could detect 1.5 *E. coli* O157:H7 per g ground beef, however, it is time consuming and the antisera is non-specific. Todd *et al.* (1988) used the HGMF in conjunction with an enzyme-labelled Ab which could yield presumptive identification within 24 h; sensitivity of the procedure was 10 cells per g of food.

Padhye and Doyle (1991) were successful in developing a sandwich enzyme-linked immunosorbant assay (ELISA) that could be completed in less than 20 h and detects 0.2 to 0.9 cells per g of food. The assay employed polyclonal Ab for capture and a monoclonal antibody (MAb) specific for EHEC of serotypes O157:H7 and O26:H11 (Padhye and Doyle, 1991a & b). A similar procedure, the dipstick immunoassay, was developed by Kim and Doyle (1992). The test had a sensitivity of 0.1 to 1.3 cells per gram in ground beef and the false-positive rate of 2.0%.

An immunostick assay specific for the somatic antigen O157 detected 100% of 35 *E. coli* O157:H7 strains tested and produced one false-positive reaction from 38 strains of other genera. Despite the high specificity, the detection limit of the assay was about 10^5 CFU/mL

(Huang and Chang, 1996). Furthermore, Milley and Sekla (1993) described a monoclonal ELISA-HGMF system against VT 1 and 2 from VTEC, including O157:H7, in stool and meat samples.

Immunomagnetic separation

The use of immunomagnetic separation (IMS) in conjunction with other detection and isolation techniques is highly recommended as the method reduces the time needed for enrichment, increases the sensitivity of detection, reduces background microflora, and is relatively inexpensive (Bennett *et al.*, 1996, Karch *et al.*, 1996). Briefly, specific antibodies are covalently bound to superparamagnetic, polystyrene beads. The beads are then mixed with an enriched food or fecal sample and incubated resulting in a bead-bacteria complex. The bead-bacteria complex is then extracted with a magnet and washed before transferring to a suitable growth media or detection system (Cudjoe *et al.*, 1993).

Weagant *et al.* (1994) recommended a regime which included enrichment of the food sample in modified tryptic soy broth (mTSB) supplemented with vancomycin, cefsulodin and cefixime (CCV-mTSB), IMS, and subsequent spread-plating on CT-SMAC. This method was sensitive to one cell per g of food. Finally, Yu and Bruno (1996) coupled IMS with electrochemoluminescence. The bead-bacteria complex was captured on the surface of a magnetized anode and light was generated.

Subtyping methods

Methods that have been used to subtype *E. coli* O157:H7 include: shiga-like toxin determination, ribotyping, plasmid profile analysis, multilocus enzyme electrophoresis, bacteriophage typing, pulsed-field gel electrophoresis (PFGE), and PCR (Griffin and Tauxe, 1991).

Although isolates collected over a defined time and single geographic location exhibit considerable diversity in plasmid content and toxin genotype (Ostroff *et al.*, 1989), multilocus electrophoresis has been used to support the theory that isolates of *E. coli* O157:H7, obtained from geographically separate outbreaks and sporadic cases of HC and HUS in North America, descend from a common clone (Whittam *et al.*,1988). Furthermore, it has been noted that O157:H7 clonal genotypes are only distantly related to other VTEC.

Barrett *et al.* (1994), suggested that phagetyping could be used as an initial screen to eliminate *E. coli* O157:H7 strains that were not of the same phage type as the outbreak strain in epidemiological investigations. In comparison to PFGE, this method is faster and less labor-intensive. PFGE, on the other hand, further discriminates strains of the same phage type, thus distinguishing non-outbreak strains from outbreak strains. Due to the lack of relationship between phage types and PFGE profiles, it is recommended to use PFGE to index strains (Krause *et al.*, 1996).

Finally, Madico *et al.* (1995) developed a simple template preparation procedure for arbitrarily primed PCR. Boiled stationary-phase cultures of *E. coli* O157:H7 were used and resulting profiles were found to be comparable with those generated from traditional DNA extraction with phenol.

Prevalence

E. coli O157:H7 and animals

As *E. coli* O157:H7 has been epidemiologically associated with the consumption of ground beef and raw milk, numerous studies have been conducted to determine the prevalence of this organism and other verotoxigenic *E. coli* in cattle, particularly dairy herds.

Wells *et al.* (1991) reported that there is no different in the recovery rates of calves <4 months old and heifers (4 to 24 months old). In a study conducted by Martin *et al.* (1994) Bob calves (<10 d old) were screened for the presence of *E. coli* O157:H7. No positive fecal swab cultures were obtained and it was concluded that the organism was not part of the normal microflora found in herds and that it may be difficult to isolate if present; 3 strains were identified as sorbitol-fermenting O157:H-.

Experimental infection of calves and adult cattle provided evidence that: 1) fecal shedding persists longer in calves than in adults, 2) *E. coli* O157:H7 is confined to the bovine intestinal tract, 3) animals can be reinfected by the same strain of *E. coli* O157:H7, 4) a high inoculation dose of farm-acquired *E. coli* O157:H7 may be required to produce fecal shedding, and 5) the majority of cattle infected with *E. coli* O157:H7 remain healthy (Wilson *et al.*, 1992; Cray and Moon, 1995; Johnson *et al.*, 1996).

In another study cited by Bettelheim (1996), VTEC was isolated in 24% (74/304) of cattle suffering from enteritis and 31% (35/113) of healthy animals. Results from both surveys supported the theory that there was no relationship between the presence of VTEC and enteritis in cattle.

In Ontario, the prevalence of VTEC in dairy cows and calves were 9.5% and 24.7%, respectively. The infection rates for some farms were as high as 60% for cows and 100% for calves; none of the isolates belonged to serogroup O157:H7 (Wilson *et al.*, 1992).

Frank *et al.* (1994) studied the colonization of *E. coli* on cattle tonsils. Two SLT-producing strains were found, neither belonged to the O157 serogroup.

E. coli O157:H7 was found in 0.28% of 3570 fecal samples from dairy cattle in 5 of 60 herds in Washington State. Furthermore, 0.71% of 1412 pastured beef fecal samples were *E. coli* O157:H7 positive; this represented 16% of 25 herds surveyed (Hancock *et al.*, 1994) . In contrast, 0.33% of 600 feedlot cattle from 2 of 20 pens were positive for the organism. Several herd management practices were identified as possible positive herd infection factors: smaller herd size, dietary constituents (less whole cottonseed in the diet), method of feeding (computerized), and a shorter period of time between pasture application of slurry and grazing. It is also evident that fecal shedding of the organism is seasonal, with the highest numbers of organisms being shed in the summer months.

Dorn (1993) postulated that the group rearing of calves and heifers during the winter months in the northern states might increase the prevalence of infected cattle and the likelihood of milk and carcass contamination at slaughter. This theory could explain the high incidence of human infections in the northern states and in the Canadian provinces.

EHEC and the rumen

The condition of animals prior to slaughter may affect the carriage of *E. coli* O157:H7. Rasmussen *et al.* (1993) demonstrated that *E. coli* O157:H7 strains grow better in the rumens of fasted cattle than in well-fed cattle. In addition, they noted that plasmids carrying antibiotic resistance, could be transferred from *E. coli* strain to *E. coli* strain. This led to the supposition that plasmids carrying virulence factors may also be transferred in this manner (Bettelheim, 1996).

Relationship of human and bovine types

While Paros *et al.* (1993) found that 90% of human isolates did not match those from cattle, Chapman and Siddons (1994) noted a strong correlation. Differences were attributed to variation in sampling and isolation techniques (Paros *et al.*, 1994; Chapman and Siddons, 1994). Wells *et al.*(1991) discovered that while infection in individual cattle was transient, herd infection could be maintained. Therefore, it could be implied that by the time an outbreak investigation has begun, the source animal may no longer be excreting the incriminated strain or another strain may be shed by the herd making it difficult to trace the source of the infection.

EHEC and chickens

Shoeni and Doyle (1994) investigated the role of chickens in *E. coli* O157:H7 infection. Shedding persisted for 10 to 11 months in one-day-old chicks inoculated with this pathogen. Moreover, the organism was isolated on the shells of eggs from infected hens.

E. coli O157:H7 in environmental samples

Boening and Tarr (1995) focused on water and soil as a source of *E. coli* O157:H7 . *E. coli* O157:H7 was detected in soil-water slurry samples spiked at "low" (10^{-8}) and "high" (10^{-2}) dilutions of *E. coli* O157:H7 at 3 and 20 days of storage at 4°C. The results suggested that water and sediment exposed to animal fecal matter may be an important vehicle for non-foodborne *E. coli* O157:H7 infections. These findings were supported by Wang *et al.* (1996). *E. coli* O157:H7 was recovered in bovine fecal samples stored at 5°C for 63 to 70 days and at 37°C up to 49 days.

E. coli O157:H7 in clinical samples.

E. coli O157:H7 is often not detected in fecal samples of patients suffering from hemorrhagic colitis and HUS. Tarr *et al.*(1990) demonstrated that the recovery rate of the organism was 100%, 91.7% and 33.3% when cultured within 2 days, between 3-6 days, and on or after 7 days after the onset of diarrhea.

E. coli in foods

E. coli O157:H7 in meat and poultry

A one year surveillance program for *E. coli* O157:H7 in ground beef obtained from plants and retail stores was conducted by the FSIS. Any product found to be contaminated by this organism was recalled. By November 1995, over 5,800 samples had been tested witth only 3 postives confirmed (Mermelstein, 1996). Doyle and Shoeni (1987) were successful in isolating *E. coli* O157:H7 from beef, pork, poultry, and lamb. In contrast, Samadpour *et al.*

(1994) found that 23% of beef, 18% of pork, 48% lamb, 12% chicken, 7% turkey, 10% fish, and 5% shellfish retail samples contained non-O157 VTEC. The identified serogroups: O6, O113 and O163 (beef) and O91 (pork) have been associated with human disease.

Behavior of *E. coli* O157:H7 in food systems

Resistance of *E. coli* O157:H7 has been documented in low-acid foods. Miller and Kaspar (1994) noted that *E. coli* O157:H7 could survive at pH 2 and 12. Furthermore, it was demonstrated that *E. coli* O157:H7 strains could survive in unpasteurized apple cider (pH range 3.7 - 4.1) with added sodium benzoate and potassium sorbate for up to 21 days. In contrast, Splittstoesser *et al.* (1996) calculated that a 4-D reduction of this organism in apple cider could be achieved at lower times and temperatures normally employed for apple juice pasteurization. Thereby, a quality product which would be safer for consumption could be offered. In another study, alcoholic fermentation of fresh cider was effective in destroying this pathogen (Semanchuk and Golden, 1996).

Abdul-Raouf *et al.*(1993) determined that acetic acid was the most effective acidulant in inhibiting *E. coli* O157:H7 growth followed by lactic and citric acid in beef slurries, respectively; this was also true of samples which were heated treated at 54°C. However, the organism was noted to survive at 5°C in beef salads containing up to 40% mayonnaise.

E. coli O157:H7 is effectively eliminated at pasteurization temperatures for fluid milk (D'Aoust *et al.*, 1988). However, the ability of the organism to survive in other dairy products has been questioned, especially cheddar cheese which can be manufactured with raw or heat-treated (non-pasteurized) milk. Cheese inoculated with 1 CFU per mL of milk was

shown to support the survival of the organism during the minimum 60 day curing period (Reitsma and Henning, 1996).

E. coli O157:H7 can survive in sausages during fermentation, drying, or subsequent storage at 4°C (Glass *et al.*, 1992). These results agree with the observations of Hinkens *et al.* (1996) who also determined that thermal processing would result in a 5 to 6 log reduction of O157:H7 in pepperoni. Moreover, the organism also survives in ground beef at least to 9 months of frozen storage at -20°C (Doyle and Schoeni, 1984).

Jackson *et al.* (1996) demonstrated that the heat resistance of *E. coli* O157:H7 is influenced by storage and holding temperatures prior to cooking. *E. coli* O157:H7 in beef patties that were frozen exhibited a greater D value compared to beef patties that were held above 0°C. However, when these patties were held at elevated temperatures prior to grilling, the heat resistance of the organisms decreased.

Economic Impact of Foodborne Disease

Economics Research Service (ERS) has estimated that *Salmonella*, *Campylobacter jejuni*, *E. coli* O157, *Staph aureus*, *Clostridium perfringens*, and *Toxoplasma gondii* cause 3.6 to 7.1 million human illness cases and 2,695 to 6,587 deaths each year in the United States. Estimated into annual costs of each bacteria are medical expenses and lost productivity which range between $5.6 to 9.4 billion. This represents a low estimation as not all pathogens were included in this study.

Estimated costs vary among pathogens because of the incidence and severity of disease. In specific, *E. coli* O157:H7 accounts for approximately 8,000 -16,000 cases of

foodborne illness and 160-400 deaths while costing 0.2-0.6 billion dollars (Buzby and Roberts, 1996). Todd (1989) estimated that *E. coli* O157:H7 infections may cost approximately $47 million in Canada and $83 million in the U.S.

Controlling *E. coli* O157:H7

Since the principle reservoir of *E. coli* O157:H7 is the intestinal tract of meat animals, raw foods of animal origin may be contaminated via feces during slaughter. The use of GMP's in meat processing and proper heat processing of foods before consumption is important. Educating food industry, food service personnel, and consumers is necessary. The FDA has published various time temperature standards for the cooking of hamburger patties (Reed, 1994). Whole beef roasts and steaks should be cooked to an internal temperature of 145° F (63°C), other cuts of meat and ground beef to 160° (71°C), ground poultry to 165° (74°C), and poultry to 180° F (82°C). The reliability of meat color as an indicator of doneness has been questioned because the meat can turn brown before reaching the target temperature. Therefore, it is recommended in the absence of a thermometer that juices should also run clear and the patty texture should be firm before consuming (FDA, 1997).

Industry intervention

Dickson and Siragusa (1994) found that water, lactic acid, and acetic acid treatments reduced the population of *E. coli* O157:H7 on lean and adipose carcass tissues. Lactic acid had more of an immediate effect, whereas acetic acid treated populations declined more gradually over a 21 day period. These trends were also observed by Hardin *et al.* (1995). In

contrast, Brackett *et al.* (1994), declared that lactic and acetic acids were ineffective due to the acid resistance demonstrated by *E. coli* O157:H7.

The use of steam and hot water spray washes have been shown to reduce microbial populations on beef tissues and may reduce the amount of trimming needed on carcass processing lines (Dorsa *et al.*, 1996, Dorsa *et al.*, 1997).

Other decontamination techniques that have been investigated include: electron and gamma irradiation, high pressure treatment, trisodium phosphate, ultrasound, high intensity light, electric and magnetic fields, air ionization, and combination treatments (physical-physical and physical-chemical) (Correy *et al.*, 1995; Earnshaw *et al.*, 1994).

Elimination of *E. coli* O157:H7 by food irradiation

Most of the work relating to the control of *E. coli* O157:H7 in foods dates after the 1992 Washington outbreak. Prior to this incident, thermal inactivation studies of *E. coli* O157:H7 were conducted in milk and ground beef (D'Aoust *et al.*, 1988; Doyle and Schoeni, 1984). Moreover, the effects of irradiation were investigated on other strains of *E. coli* (Monk *et al.*, 1995).

In the first report detailing the effects of ionizing radiation on *E. coli* O157:H7, Thayer and Boyd (1993) determined the irradiation (γ) sensitivity of *E. coli* O157:H7 in steak tartare (ground lean top round beef) and studied the influence of irradiation dose, temperature, and atmosphere in mechanically deboned chicken meat (MDCM). Under the same conditions, there was no difference between *E. coli* O157:H7 sensitivity in ground beef (2.6% fat) and MDCM (21.3% fat). With respect to MDCM, no effect was observed when samples were

irradiated under vacuum or air. Sixteen hour stationary-phase cultures (0.27 kGy) were more radiation-resistant than 4 h log-phase cultures (0.16 kGy). Also, *E. coli* O157:H7 in samples kept at -5°C were more resistant that those kept at 5°C (0.44 and 0.28 kGy, respectively). Finally, no viable cells or verotoxins were detected in ground beef that was inoculated with 4.8 $\log_{10}$ CFU/g of *E. coli* O157:H7, irradiated at 1.5 kGy at 0°C, and subjected to 20 h temperature abuse at 35°C. The authors concluded that irradiation with a minimum dose of 1.5 kGy and a 3.0 kGy maximum would offer substantial protection to consumers.

In a subsequent study, Thayer *et al.* (1995) found that *E. coli* O157:H7 D_{10} values (~0.30 kGy) did not vary with the suspending meat: beef, pork, lamb, turkey breast, and turkey leg meat.

Clavero *et al.* (1995) studied the effects of fat content and temperature on a D_{10} values of stationary-phase *E. coli* O157:H7. D_{10} values were significantly higher for those samples irradiated in frozen condition (-17 to -15°C) than under refrigeration (3 to 5°C) temperature; however, fat content (8-14% [low fat] and 27-28% [high fat]) did not have an effect. D_{10} values ranged from 0.251 kGy in refrigerated ground beef and 0.307 in frozen ground beef. Based on the highest D_{10} value obtained for this organism, it was theorized that an applied dose of 2.5 kGy would be sufficient to inactivate 8.1 $\log_{10}$ *E. coli* O157:H7.

The results of Fu *et al.* (1995) indicated that dose rates did not affect the sensitivity of *E. coli* O157:H7 to electron beam irradiation. In addition, there was no differences in D_{10} values between steaks packaged under air or vacuum; their findings agreed with Thayer and Boyd (1993). In ground beef, cell counts decreased after irradiation at 0.8 kGy with subsequent storage for 7 days at 7°C; however, rapid growth proceeded when samples were

subjected to abuse temperatures (25°C) at day 7-9. Furthermore, doses of 1.5 and 2.0 kGy

was shown to decrease the population of this pathogen significantly in steaks and ground beef,

respectively.

References

Abdul-Raouf, U.M., L.R. Beuchat, and M.S. Ammar. 1993. Survival and growth of *Escherichia coli* O157:H7 in ground, roasted beef as affected by pH, acidulants, and temperature. Appl. Environ. Microbiol. 59:2364-2368.

ADA. 1996. Position of the American Dietitic Association: food irradiation. J. Am. Diet. Assoc. 96:69-72.

Ahmed, N.M., and D.E. Connor. 1995. Evaluation of various media for recovery of thermally-injured *Escherichia coli* O157:H7. J. Food Prot. 58:357-360.

Alur, M.D. S.P. Chawla, and P.M. Nair. 1992. Microbiological method to identify irradiated meat. J. Food Sci. 57:593-595.

Anellis, A., D. Berkowitz, and D. Kemper. 1973. Comparative resistance of nonsporogenic bacteria to low-temperature gamma irradiation. Appl. Microbiol. 25:517-523.

Barna, J. 1979. Compilation of bioassay data on the wholesomeness of irradiated food items. Acta Aliment. 8:205-315.

Barrett, T.J., J.B. Kaper, A.E. Jerse, and I.K. Wachsmuth. 1992. Virulence factors in shiga-like toxin-producing *Escherichia coli* isolated from humans and cattle. J. Infect. Dis. 165:979-980.

Barrett, T.J., H. Lior, J.H. Green, R. Khakhria, J.G. Wells, B.P. Bell, K.D. Greene, J. Lewis, and P.M. Griffin. 1994. Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157:H7 by using pulsed-field gel electrophoresis and phage typing. J. Clin. Microbiol. 32:3013-3017.

Bean, N.H., and P.M. Griffin. 1990. Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends. J. Food Prot. 53:804-817.

Bennett, A.R., S. MacPhee, and R.P. Betts. 1996. The isolation and detection of *Escherichia coli* O157 by use of immunomagnetic separation and immunoassay procedures. Letters Appl. Microbiol. 22:237-243.

Bennett, A.R., S. MacPhee, and R.P. Betts. 1995. Evaluation of methods for the isolation and detection of *Escherichia coli* O157 in minced beef. Letters Appl. Microbiol. 20:375-379.

Bettelheim, K.A. 1996. Enterohemorrhagic *Escherichia coli*: a new problem, an old group of organisms. Aust. Vet. J. 73:20-26.

Beutin, L., D. Geier, S. Zimmermann, and H. Karch. 1995. Virulence markers of shiga-like toxin-producing *Escherichia coli* strains originating from healthy domestic animals of different species. J. Clin. Microbiol. 33:631-635.

Beutin, L., M.A. Montenegro, I. Orskov, F. Orskov, J. Prada, S. Zimmermann, and R. Stephan. 1989. Close association of verotoxin (shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J. Clin. Microbiol. 27:2559-2564.

Blank, G., and D. Corrigan. 1995. Comparison of resistance of fungal spores to gamma and electron beam radiation. Int. J. Food Microbiol. 26:269-277.

Boening, D.W., and P.I. Tarr. 1995. Proposed method for isolation of *Escherichia coli* O157:H7 from environmental samples. J. Environ. Health. 57:19-21.

Boyd, D.R., A.V.J. Crone, J.T.G. Hamilton, M.V. Hand, M.H. Stevenson, and P.J. Stevenson. 1991. Synthesis, characterization, and potential use of 2-dodecylcyclobutanone as a marker for irradiated chicken. J. Agric. Food Chem. 39:789-792.

Brackett, R.E., Y.Y. Hao, and M.P. Doyle. 1994. Ineffectiveness of hot acid sprays to decontaminate *Esherichia coli* O157:H7 on beef. J. Food Prot. 57:198-203.

Bruhn, C. M. 1995. Strategies for communicating the facts on food irradiation to consumers. J. Food Prot. 58:213-216.

Bruhn, C.M., and H.G. Schutz. 1989. Consumer awareness and outlook for acceptance of food irradiation. Food Technol. :93-94,97.

Buzby, J., and T. Roberts. 1996. Microbial foodborne illness. Choices. First Quarter: 14-17.

CAST. 1986. Ionizing energy in food processing and pest control: I. Wholesomeness of food treated with ionizing energy. Task Force Report No. 109. Council; for Agricultural Science and Technology, Ames, A.

CDC. 1997. Outbreaks of *Escherichia coli* O157:H7 Infection and Cryptosporidiosis Associated with Drinking Unpasteurized Apple Cider--Connecticut and New York, October 1996. Morbid. Mortal. Weekly Rep. 46:4-8.

CDC. 1996. Outbreak of Escherichia coli O157:H7 infections associated with drinking unpasteurized commercial apple juice British Columbia, California, Colorado, and Washington, October 1996. Morbid. Mortal. Weekly Rep. 45:975.

CDC. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami - Washington and California, 1994. Morbid. Mortal. Weekly Rep. 44:157-160.

CDC. 1994. *Escherichia coli* O157:H7 outbreak linked to home-cooked hamburger - California, July 1993. Morbid. Mortal. Weekly Rep. 43:213-216

CDC. 1991. Foodborne outbreak of gastroenteritis caused by *Escherichia coli* O157:H7 - North Dakota, 1990. Morbid. Mortal. Weekly Rep. 40:265-267.

Chapman, P.A., and C.A. Siddons. 1994. A comparison of strains of *Escherichia coli* O157:H7 from human and cattle in Sheffield, United Kingdom. J. Infect. Dis. 170:251-252.

Chart, H., O.A. Okubadejo, and B. Rowe. 1992. The serological relationship between *Escherichia coli* O157 and *Yersinia enterocolitica* O9 using sera from patients with brucellosis. Epidemiol. Infect. 108:77-85.

Chart, H., T. Cheasty, D. Cope, R.J. Gross, and B. Rowe. 1991. The serological relationship between *Yersinia enterocolitica* O9 and *Escherichia coli* O157 using sera from patients with yersiniosis and haemolytic uraemic syndrome. Epidemiol. Infect. 107:349-356.

Chuaqui-Offermanns, N., and T. McDougall. 1991. An HPLC method to determine *o*-tyrosine in chicken meat. J. Agric. Food Chem. 39:300-302.

Clavero, M.R.S., and L.R. Beuchat. 1995. Suitability of selective plating media for recovering heat- or freeze-stressed *Escherichia coli* O157:H7 from tryptic soy broth and ground beef. Appl. Environ. Microbiol. 61:3268-3273.

Clavero, M.R.S., J.D. Monk, L.R. Beuchat, M.P. Doyle, and R.E. Brackett. 1994. Inactivation of *Escherichia coli* O157:H7, salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. 60: 2069 - 2075.

Correy, J.E.L., C. James, S.J. James, and M. Hinton. 1995. *Salmonella, Campylobacter* and *Escherichia coli* O157:H7 decontamination techniques for the future. Food Microbiol. 28:187-196.

Crawford, Y.J., E.A. Murano, D.G. Olson, and K. Shenoy. 1996. Use of high hydrostatic pressure and irradiation to eliminate *Clostridium sporogenes* spores in chicken breast. J. Food Prot. 59:711-715.

Cray Jr., W.C., and H.W. Moon. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 61:1586-1590.

Cubbon, M.D., J.E. Coia, M.F. Hanson, and F. M. Thomson-Carter. 1996. A comparison of immunomagnetic separation, direct culture and polymerase chain reaction for the detection of verocytotoxin-producing *Escherichia coli* O157 in human faeces. J. Med. Microbiol. 44:219-222.

Cudjoe, K.S., P.D. Patel, E. Olsen, E. Skjerve, and O. Olsvik. 1993. Immunomagnetic separation techniques for the detection of pathogenic bacteria in foods. In: New Techniques in Food and Beverage Microbiology.

D'Aoust, J.Y., C.E. Park, R.A. Szabo, E.C.D. Todd, D.B. Emmons, and R.C. McKellar. 1988. Thermal inactivation of *Campylobacter* species, *Yersinia enterocolitica*, and hemorrhagic *Escherichia coli* O157:H7 in fluid milk. J. Dairy Sci. 71:3230-3236.

Dempster, J.F. 1985. Radiation preservation of meat and meat products: a review. Meat Sci. 12:61-89.

Deng, M.Y., and P.M. Fratamico. 1996. A multiplex PCR for rapid identification of Shiga-like toxin-producing *Escherichia coli* O157:H7 isolated from foods. J. Food Prot. 59:570-576.

Desrosiers, M.F. 1991. Electron spin resonance for monitoring radiation-processed meats containing bones. J. Food Sci. 56:1104-1105.

Dickson, J.S., and R.B. Maxcy. 1985. Irradiation of meat for the production of fermented sausage. J. Food Sci. 50:1007-1009,1013.

Dickson, J.S., and G.R. Siragusa. 1994. Survival of *Salmonella typhimurium, Escherichia coli* O157:H7 and *Listeria monocytogenes* during storage on beef sanitized with organic acids. J. Food Saf. 14:313-327.

Diehl, D.F. 1995. Safety of irradiated foods. Marcel Dekker, Inc., New York, NY.

Dodd, N.J.F., J. Haishun, J.S. Lea, and A.J. Swallow. 1992. Factors influencing the yield of free radicals in irradiated chicken bones. Int. J. Food Sci. Technol. 27:371-383.

Dorn, C.R. 1993. Review of foodborne outbreak of *Escherichia coli* O157:H7 infection in the western United States. JAVMA. 203:1583-1587.

Dorsa, W.J., C.N. Cutter, and G.R. Siragusa. 1997. Effects of steam-vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. J. Food Prot. 60:114-119.

Dorsa, W.J., C.N. Cutter, G.R. Siragusa, and M. Koohmaraie. 1996. Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. J. Food Prot. 59:127-135.

Doyle, M.P. 1991. *Escherichia coli* O157:H7 and its significance in foods. Food. 12:289-301.

Doyle, M.P., and J.L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394-2396.

Doyle, M.P., and J.L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. 48:855-856.

Earnshaw, R.G., J. Appleyard, and R.M. Hurst. 1995. Understanding physical inactivation processes: combined preservation opportunities using heat, ultrasound and pressure. Int. J. Food. Microbiol. 28:197-219.

Euro Surveillance. 1997. Scottish outbreak of *Escherichia coli O157:H7*, November - December 1996. http://www.b3e.jussieu.fr/ceses/EuroSurv/v2n1/en7-221.html.

FDA. 1997. Addendum (February 1997) FSIS Recommendations for Cooking Ground Beef. http://www.usda.gov/agency/fsis.

FDA. 1994. Filing of food additive petition. Notice. U.S. Food and Drug Administration. Fed. Regist. 59:43848-43849.

Frank, G.H., R.E. Briggs, and R.A. Schneider. 1994. Characterization of *Escherichia coli* isolated from the tonsils of cattle. J. Clin. Microbiol. 32:256-258.

Fu, A., J.G. Sebranek, and E.A. Murano. 1995. Survival of *Listeria monocytogenes, Yersinia enterocolitica* and *Escherichia coli* O157:H7 and quality changes after irradiation of beef steaks and ground beef. J. Food Sci. 60:972-977.

Glass, K.A., J.M. Loeffelholz, J.P. Ford, and M.P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol. 58:2513-2516.

Goldblith, S.A. 1966. Historical development of food irradiation, SM-73/54. Proc. Int. Symp. on Food Irradiation. International Atomic Energy Agency STI/PUB/127, Vienna, Austria.

Grant, I., and M. F. Patterson. 1995. Combined effect of gamma radiation and heating on the destruction of *Listeria monocytogenes* and *Salmonella typhimurium* in cook-chill roast beef and gravy. Int. J. Food Microbiol. 27:117-128.

Grant, I., and M. F. Patterson. 1991. Effect of irradiation and modified atmosphere packaging on the microbiological and sensory quality of pork stored at refrigeration temperatures. Int. J. Food Sci. Technol. 26: 507-519.

Griffin, P.M., and R.V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60-98.

Gunzer, F., H. Bohm, H. Russmann, M. Bitzan, S. Aleksic, and H. Karch. 1992. Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. J. Clin. Microbiol. 30:1807-1810.

Hancock, D.D., T.E. Besser, M.L. Kinsel, P.I. Tarr, D.H. Rice, and M.G. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect. 113:199-207.

Hardin, M.D., G.R. Acuff, L.M. Lucia, J.S. Oman, and J.W. Savell. 1995. Comparison of methods for decontamination from beef carcass surfaces. J. Food Prot. 58:368-374.

Hashim, I.B., A.V.A. Resurreccion, and K.H. McWatters. 1995. Consumer acceptance of irradiated poultry. Poul. Sci. 74:1287-1294.

Hinkens, J.C., N.G. Faith, T.D. Lorang, P. Bailey, D. Buege, C.W. Kaspar, and J.B. Luchansky. 1996. Validation of pepperoni processes for control of *Escherichia coli* O157:H7. J. Food Prot. 59:1260-1266.

Huang, S.W., and T.C. Chang. 1996. Specific identification of *Escherichia coli* O157 by an immunostick method using commercially available antibodies. J. Food Prot. 59:670-674.

Huck, L.G., C.R. Dorn, and E.J. Angrick. 1995. DNA probe for detection of serogroup O157 enterohemorrhagic *Escherichia coli*. Int. J. Food Microbiol. 25:277-287.

IASR. 1996. Outbreaks of enterohemorrhagic *Escherichia coli* O157:H7 infection, 1996, Japan. http://www. nih.go.jp:80/yoken/iasr.

Jackson, T.C., M.D. Hardin, and G.R. Acuff. 1996. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temoperatures. J. Food Prot. 59:230-237.

Jay, J.M. 1992. Modern Food Microbiology. Van Nostrand Reinhold Co., New York. Jinneman, K.C., P.A. Trost, W.E. Hill, S.D. Weagent, J.L. Bryant, C.A. Kaysner, and M.M. Wekell. 1995. Comparison of template preparation methods from foods for amplification of *Escherichia coli* O157 shiga-like toxins type I and II DNA by multiplex polymerase chain reaction. J. Food Prot. 58:722-726.

Johnson, J.L., B.E. Rose, A.K. Sharar, G.M. Ransom, C.P. Lattuada, and A.M. McNamara. 1995. Methods used for detection and recovery of *Escherichia coli* O157:H7 associated with a food-borne disease outbreak. J. Food Prot. 58:597-603.

Johnson, R.P., W.C. Cray Jr., and S.T. Johnson. 1996. Serum antibody responses of cattle following experimental infection with *Escherichia coli* O157:H7. Infect. Imm. 64:1879-1883.

Karch, H., H. Bohm, H. Schmidt, F. Gunzer, S. Aleksic, and J. Heesemann. 1993. Clonal structure and pathogenicity of shiga-like toxin-producing, sorbitol-fermenting *Escherichia coli* O157:H-. J. Clin. Microbiol. 31:1200-1205.

Karch, H., C. Janetzki-Mittmann, S. Aleksic amd M. Datz. 1996. Isolation of enterohemmhoragic *Escherichia coli* O157 strains from patients with hemolytic-uremic syndrome by using immunomagnetic separation, DNA-based methods, and direct culture. J. Clin. Microbiol. 34:516-519.

Kim, M.S., and M.P. Doyle. 1992. Dipstick immunoassay to detect enterohemorrhagic *Escherichia coli* O157:H7 in retail ground beef. Appl. Environ. Microbiol. 58:1764-1767.

Konowalchuk, J., J.I. Speirs, and S. Stavric. 1977. Vero response to a cytotoxin of *Escherichia coli*. Infect. Imm. 18:775-779.

Kornacki, J.L., and E.H. Marth. 1982. Foodborne illness caused by *Escherichia coli*: a review. J. Food Prot. 45:1051-1067.

Krause, U., F.M. Thomson-Carter, and T.H. Pennington. 1996. Molecular epidemiology of *Escherichia coli* O157:H7 by pulsed-field gel electrophoresis and comparison with that by bacteriophage typing. J. Clin. Microbiol. 34:959-961.

Levine, M.M. 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Infect. Dis. 155:377-389.

Lior, H. 1994. *Escherichia coli* O157:H7 and verotoxigenic *Escherichia coli* (VTEC). Dairy Food Environ. San. 14:378-382.

Loaharanu, P. 1994. Status and prospects of food irradiation. Food Technol. 48:124-131.

Loaharanu, P. 1989. International trade in irradiated foods: regional status and outlook. Food Technol. 43:77-80.

Louie, M., J. De Azavedo, R. Clarke, A. Borczyk, H. Lior, M. Richter, and J. Brunton. 1994. Sequence heterogeneity of the *eae* gene and detection of verotoxin-producing *Escherichia coli* using serotype-specific primers. Epidemiol. Infect. 112:449-461.

MacLeod. D.L., and C.L. Gyles. 1989. Effects of culture conditions on yield of Shiga-like toxin-IIv from *Escherichia coli*. Can. J. Microbiol. 35:623-629.

Madico, G., N.S. Akopyants, and D.E. Berg. 1995. Arbitrarily primed PCR DNA fingerprinting of *Escherichia coli* O157:H7 strains by using templates from boiled cultures. J. Clin. Microbiol. 33:1534-1536.

Marcotte, M. 1992. Irradiated strawberries enter the U.S. market. Food Technol. 46:80-86.

Martin, D.R., P.M. Uhler, A.J.G. Okrend, and J.Y. Chiu. 1994. Test of Bob Calf fecal swabs for the presence of *Escherichia coli* O157:H7. J. Food Prot. 57:70-72.

Mermelstein, N.H. 1996. Testing for *E. coli* O157:H7 in meat. Food Technol. 50:36.

Miller, L.G., and C.W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J. Food Prot. 57:460-464.

Milley, D.G., and L.H. Sekla. 1993. An enzyme-linked immunosorbent assay-based isolation procedure for verotoxigenic *Escherichia coli*. Appl. Environ. Microbiol. 59:4223-4229.

Molenda, J.R. 1994. *Escherichia coli* (including O157:H7): an environmental health perspective. Dairy Food Environ. Sanit. 14:742-747.

Monk, J.D., L.R. Beuchat, and M.P. Doyle. 1995. Irradiation inactivation of food-borne microorganisms. J. Food Prot. 58:197-208.

Morgan, D., C.P. Newman, D.N. Hutchinson, A.M. Walker, B.Rowe, and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157:H7 infections associated with the consumption of yoghurt. Epidemiol. Infect. 111:181-187.

Morgan, G.M., C. Newman, S.R. Palmer, J.B. Allen, W. Shepherd, A.M. Rampling, R.E. Warren, R.J. Gross, S.M. Scotland, and H.R. Smith. 1988. First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157 in the UK. Epidemiol. Infect. 101:83-91.

Murano, E.A. 1995. Irradiation processing, Ch.2. *In* E.A. Murano (ed.), Food irradiation: a sourcebook. Iowa State University Press, Ames, IA.

Murano, P.S. 1995. Irradiation processing, Ch.3. *In* E.A. Murano (ed.), Food irradiation: a sourcebook. Iowa State University Press, Ames, IA.

Neill, M.A. 1994. *E. coli* O157:H7 time capsule: what do we know and when did we know it. Dairy Food Environ. Sanit. 14:374-377.

Neill, M.A. 1989. *E. coli* O157:H7 - current concepts and future prospects. J. Food Saf. 10:99-106.

Notermans, S., K. Wernars, P.S. Soentoro, J. Dufrenne, and W. Jansen. 1991. DNA hybridization and latex agglutination for detection of heat-labile and shiga-like toxin-producing *Escherichia coli* in meat. Int. J. Food Microbiol. 13:31-40.

O'Brien, A.D., T.A. Lively, M.E. Chen, S.W. Rothman, and S.B. Formal. 1983. *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a shigella dysenteriae I (shiga) like cytotoxin. Lancet. i:702.

Okrend, A.J.G., B.E. Rose, and B. Bennett. 1990a. A screening method for the isolation of *Escherichia coli* O157:H7 from ground beef. J. Food Prot. 53:249-252.

Okrend, A.J.G., B.E. Rose, and C.P. Lattuada. 1990b. Use of 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide in MacConkey Sorbitol Agar to aid in the isolation of *Escherichia coli* O157:H7 from ground beef. J. Food Prot. 53:941-943.

Olson, D.G. 1995. Irradiation processing, Ch.1. *In* E.A. Murano (ed.), Food irradiation: a sourcebook. Iowa State University Press, Ames, IA.

Ostroff, S.M., P.I. Tarr, M.A. Neill, J.H. Lewis, N. Hargrett-Bean, and J.M. Kobayashi. 1989. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J. Infect. Dis. 160:994-998.

Padhye, N.V., and M.P. Doyle. 1992. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. J. Food Prot. 55:555-565.

Padhye, N.V., and M.P. Doyle. 1991a. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl. Environ. Microbiol. 57:2693-2698.

Padhye, N.V., and M.P. Doyle. 1991b. Production and characterization of a monoclonal antibody specific for the enterohemorrhagic *Escherichia coli* of serotypes O157:H7 and O26:H11. J. Clin. Microbiol. 29:99-103.

Palumbo, S.A., J.E. Call, F.J. Schultz, and A.C. Williams. 1995. Minimum and maximum temperatures for growth and verotoxin production by hemorrhagic strains of *Escherichia coli*. J. Food Prot. 58:352-356.

Paros, M., P.I. Tarr, H. Kim, T.E. Besser, and D.D. Hancock. 1993. A comparison of human and bovine *Escherichia coli* O157:H7 isolates by toxin genotype, plasmid profile, and bacteriophage λ-restriction fragment length polymorphism profile. J. Infect. Dis. 168:1300-1303.

Patterson, M. 1989. Sensitivity of *Listeria monocytogenes* to irradiation on poultry meat and in phosphate-buffered saline. Lett. Appl. Microbiol. 8:181-184.

Patterson, R.L.S., and M.H. Stevenson. 1995. Irradiation-induced off-odour in chicken and its possible control. Br. Poul. Sci. 36:425-441.

Pszczola, D. 1993. Irradiated poultry makes U.S. debut in Midwest and Florida markets. Food Technol. 46:89-92,94,96.

Raghubeer, E.V., and J.R. Matches. 1990. Temperature range for growth of *Escherichia coli* serotype O157:H7 and selected coliforms in *E. coli* medium. J. Clin. Microbiol. 28:803-805.

Rasmussen, M.A., W.C. Cray, Jr., T.A. Casey, and S. C. Whipp. 1993. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. FEMS Microbiol. Lett. 114:79-84.

Reed, G.H. 1994. Foodborne illness (part 8) *Escherichia coli*. Dairy Food Environ. Sanit. 14:329-330.

Resurreccion, A.V.A., F.C.F. Galvez, S.M. Fletcher, and S.K. Misra. 1995. Consumer attitudes toward irradiated food: results of a new study. J. Food Prot. 58:193-196.

Retsma, C.J., and D.R. Henning. 1996. Survival of enterohemorrhagic *Escherichia coli* O157:H7 during the manufacture and curing of cheddar cheese. J. Food Prot. 59:460-464.

Rice, E.W., E.G. Sowers, C.H. Johnson, M.E. Dunnigan, N.A. Strockbine, and S.C. Edberg. 1992. Serological cross-reactions between *Escherichia coli* O157 and other species of the genus *Escherichia*. J. Clin. Microbiol. 30:1315-1316.

Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Hebert, E.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New Engl. J. Med. 12:681-685.

Sahasrabudhe, M.R., C. Willemot, J. Vanderstoep, H. Vander Pol, and P. Townsend. AIC/CIFST joint statement on food irradiation. J. Inst. Can. Sci. Technol. Aliment. 22:190-196.

Samadpour, M., G.E. Ongerth, J. Liston, N. Tran, D. Nguyen, T.S. Whittam, R.A. Wilson, and P.I. Tarr. 1994. Occurrence of shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, Washington. Appl. Environ. Microbiol. 60:1038-1040.

Satin, M. 1993. Food Irradiation A Guidebook. Technomic, Lancaster, Pennsylvania.

Schoeni, J.L., and M.P. Doyle. 1994. Variable colonization of chickens perorally inoculated with *Escherichia coli* O157:H7 and subsequent contamination of eggs. Appl. Environ. Microbiol. 60:2958-2962.

Scotland, S.M., H.R. Smith, and B. Rowe. 1985. Two distinct toxins active on vero cells from *Escherichia coli* O157. Lancet. ii:885-886.

Scotland, S.M., G.A. Willshaw, H.R. Smith, and B. Rowe. 1987. Properties of strains of *Escherichia coli* belonging to serogroup O157 with special refernce to production of Vero cytotoxins VT 1 and VT 2. Epidemiol. Infect. 99:613-625.

Semanchuk, J.J., and D.A. Golden. 1996. Survival of *Escherichia coli* O157:H7 during fermentation of apple cider. J. Food Prot. 59:1256-1259.

Splittstoesser, D.F. M.C. McLellan, and J.J. Churey. 1996. Heat resistance of *Escherichia coli* O157:H7 in apple juice. J. Food Prot. 59:226-229.

Tarr, P.I., M.A. Neill, C.R. Claussen, S.L. Watkins, D.L. Christie, and R.O. Hickman. 1990. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome: importance of early cultures in establishing etiology. J. Infect. Dis. 162:553-556.

Tarté, R. 1996. Sensitivity of *Listeria* to irradiation in raw ground meat, as affected by type of radiation, product temperature, packaging atmosphere, and recovery medium. Ph.D. Dissertation. Iowa State University, Ames, IA.

Tarté R., R, E.A. Murano, and D.G. Olson. 1996. Survival and injury of *Listeria monocytogenes*, *Listeria innocua* and *Listeria ivanovii* in ground pork following electron beam irradiation. J. Food Prot. 59:596-600.

Tarté, R., D.G. Olson, E.A. Murano, and P. Sundaram. 1996. Sensitivity of *Listeria monocytogenes* to irradiation in ground beef, as affected by type of radiation (x-rays or electrons), product temperature, packaging atmosphere, and recovery medium. (Paper accepted to the Journal of Food Science).

Thayer, Donald W. 1993. Extending shelf life of poultry and red meat by irradiation processing.. J. Food Prot. 56: 831 - 833.

Thayer, D.W., and G. Boyd. 1993. Elimination of *Escherichia coli* O157:H7 in meats by gamma irradiation. Appl. Environ. Microbiol. 59:1030-1034.

Thayer, D.W., G. Boyd, J.B. Fox, Jr., L. Lakritz, and J.W. Hampton. 1995. Variations in radiation sensitivity of foodborne pathogens associated with the suspending meat. J. Food Sci. 60:63-67.

Todd, E.C.D. 1989. Costs of acute bacterial foodborne disease in Canada and the United States. Int. J. Food. Microbiol. 9:313-326.

Todd, E.C.D. 1988. Foodborne and waterborne disease in Canada - 1982 annual summary. J. Food Prot. 51:56-65.

Todd, E.C.D., R.A. Szabo, P. Peterkin, A.N. Sharpe, L. Parrington, D. Bundle, M.A.J. Gidney, and M.B. Perry. 1988. Rapid hydrophobic grid membrane filter-enzyme-labeled antibody procedure for identification and enumeration of *Escherichia coli* O157 in foods. Appl. Environ. Microbiol. 54:2536-2540.

Urbain, W.M. 1986. Food Irradiation. Academic Press, Inc., Orlando, FL.

Wang, G., T. Zhao, and M.P. Doyle. 1996. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. Appl. Environ. Microbiol. 62:2567-2570.

WHO. 1988. Food irradiation a technique for preserving and improving the safety of food. World Health Organization, Geneva, Switzerland.

Wilson, I.G., A. Gilmour, and J.E. Cooper. 1993. New Techniques in Food and Beverage Microbiology.

Weagant, S.D., J.L. Bryant, and K.G. Jinneman. 1995. An improved rapid technique for isolation of *Escherichia coli* O157:H7 from foods. J. Food Prot. 58:7-12.

Wells, J.G., B.R. Davis, I.K. Wachsmuth, L.W. Riley, R.S. Remis, R. Sokolow, and G.K. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J. Clin. Microbiol. 18:512-520.

Wells, J.G., L.D. Shipman, K.D.Greene, E.G. Sowers, J.H. Green, D.N. Cameron, F.P. Downes, M.L. Martin, P.M. Griffin, S.M. Ostroff, M.E. Potter, R.V. Tauxe, and I.K. Wachsmuth. 1991. Isolation of *Escherichia coli* serotype O157:H7 and other shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. 29:985-989.

Whittam, T.S., I.K. Wachsmuth, and R.A. Wilson. 1988. Genetic evidence of clonal descent of *Escherichia coli* O157:H7 associated with hemorrhagic colitis and hemolytic uremic syndrome. J. Infect. Dis. 157:1124-1133.

Wilson, J.B., S.A. McEwen, R.C. Clarke, K.E. Leslie, R.A. Wilson, D. Waltner-Toews, and C.L. Gyles. 1992. Distribution and characteristics of verocytotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. Epidemiol. Infect. 108: 423-439.

Yu, H., and J.G. Bruno. 1996. Immunomagnetic-electrochemiluminescent detection of *Escherichia coli* O157 and *Salmonella typhimurium* in foods and environmental water samples. Appl. Environ. Microbiol. 62:587-592.

Zadik, P.M., P.A. Chapman, and C.A. Siddons. 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. J. Med. Microbiol. 39:155-158.

CHAPTER 3. SENSITIVITY AND SURVIVAL OF *ESCHERICHIA COLI* O157:H7 ISOLATES AFTER X-RAY AND ELECTRON BEAM IRRADIATION IN RAW GROUND MEAT

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ABSTRACT

The irradiation sensitivity of 25 bovine *Escherichia coli* O157:H7 isolates along with a

Washington outbreak strain and an enterotoxigenic strain was determined. A cocktail of the

five most resistant strains, as calculated by an average of D-values over 3 repetitions, was then

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inoculated into ground beef (15.0 - 19.5% fat) and subjected to a minimum surface dose of 1.5 kGy. Samples were analyzed on day 0, 5, 10, 20, and 30 for the presence/absence of *E.* Results indicated that there were differences in irradiation sensitivity among isolates (p < .001); mean D_{10} values ranged from 0.09 to 0.37 kGy. In addition, *E.coli* O157:H7 survivors were recovered throughout the storage study indicating that a higher minimum dose of irradiation may be needed for non-frozen comminuted meat products that are heavily contaminated (10^3 - 10^4 cells/g) with this pathogen.

INTRODUCTION

Since 1982, *E. coli* O157:H7 has been recognized as a foodborne pathogen (47). Although this organism has been mainly associated with the consumption of undercooked ground meat, sporadic outbreaks have linked *E. coli* O157:H7 with a variety of vehicles (37,38,40). Recently, outbreaks have been reported in Japan (31), the western United States and British Columbia (9), Connecticut (8), and Scotland (24) involving raw beef livers, unpasteurized apple juice, apple cider, and cooked meat, respectively.

In light of the 1993 west coast outbreak, much research has been conducted on studying the prevalence (22,29,35,57,59) and virulence of the organism (3,5,34,43,49,50). Research conducted by the National Animal Disease Center in Ames, Iowa demonstrated that 25 *E. coli* O157:H7 isolates, obtained from a national prevalence study in dairy cattle, differed from each other in their resistance to antibiotics (14).

Proper cooking and pasteurization effectively destroy this pathogen (16,21,32,42). Other methods to control *E. coli* O157:H7 in meat products have been examined. Steam, hot

waters sprays and organic acids such as lactic and acetic can reduce, but not eliminate, populations of *E. coli* O157:H7 (6,17,19,20).

The usefulness of food irradiation has been recognized for many decades. One of the first experiments demonstrating its preservation capacities was the irradiation of hamburger meat in 1943 (27). Despite scientific support and expert endorsement efforts (1,2,7,58), its acceptance has been delayed due to regulatory aspects, equipment availability, and the unfamiliarity of consumers with this process. In the U.S., the irradiation of pork has been permitted at 0.3 to 1.0 kGy for *Trichinella spiralis* inactivation since 1985 (23) and in 1992, poultry was approved for irradiation treatment at 1.5 to 3 kGy for pathogen control (45). Currently, the FDA is reviewing a petition to allow the irradiation of fresh/chilled and frozen red meats at 1.5 to 4.5 kGy and 2-3 to 7 kGy, respectively (25).

It was the purpose of this study, therefore, to determine whether these 25 strains differed in their resistance to processing treatments as well, in specific to irradiation. In addition, the adequacy of the minimum surface dose of 1.5 kGy in eliminating a cocktail of the most radiation-resistant strains was assessed.

MATERIALS AND METHODS

1. Sensitivity of E. coli O157:H7 isolates to X-ray irradiation

Cultures

The organisms studied are shown in Table 1. Cultures were maintained on TSA slants (Difco Laboratories, Detroit, MI) and stored at 4°C. When needed, a loopful of culture was

transferred to 10 mL tryptic soy broth (TSB) (Difco) for 24 h at 37°C, followed by another transfer to obtain a 24 h stationary phase culture.

Ground beef

Ground lean beef (9.1 - 13.8% fat) was obtained from the Iowa State University Meat Laboratory. The meat was divided into ~100 g portions, vacuum packaged, irradiated at 35 kGy to achieve sterility, and stored at -20°C until needed.

Sample preparation

Sterile ground beef was thawed at room temperature overnight. One hundred gram portions were placed in Stomacher bags (Seward Medical, London, UK). Four mL of the 24 h bacterial suspension was added to 100 g of the sterile beef (for an inoculum concentration of approximately 4×10^7 cells/g) and blended with a Stomacher 400 Lab Blender (Seward Medical) for 2 min. Portions of inoculated meat (25g) were packed into 60 x 15 mm polystyrene petri dishes (Fisher Scientific Co., Pittsburgh, PA) and the lids were secured by wrapping Parafilm laboratory film (American Can Co., Neenah, WI) around the circumference of the dishes. Samples were immediately irradiated.

Irradiation and dosimetry

Samples were irradiated with a CICRE III Linear Electron Accelerator (MeV Industrie S.A., Jouy-en-Josas, France) located at the Iowa State University Linear Accelerator Facility. Samples were subjected to doses between 0.0 (control) and 0.86 kGy of X-ray irradiation

(0.067 kGy/min) at ambient temperature. X-rays were generated by placing a stainless steel target in the path of the electron beam (5 MeV, 12 kW).

To determine absorbed doses, dosimeter alanine pellets (Bruker Analytische Messteknik, Rheinstetten, Germany), calibrated and traceable to a national standard, were by placed on the top and bottom of one of the petri dishes. Immediately following irradiation, absorbed doses were determined by electron paramagnetic resonance (EPR) on a Bruker EMS 104 EPR Analyzer. The average of the top and bottom readings were taken as the average absorbed dose.

Determination of survivors

Immediately after irradiation, samples were diluted 1:10 in 0.1% peptone diluent (Difco) and homogenized with the Stomacher Blender for 2 min. Serial dilutions in 0.1% peptone were then prepared and 0.1 mL volumes of the appropriate dilution were surface plated on TSA in duplicate. Plates were incubated aerobically for 24 h at 37°C. After incubation, colonies were counted and recorded as the average of duplicate plates in colony forming units per gram (CFU/g) of sample.

Calculation of D_{10} values

D_{10} values were determined by plotting the log survivors (CFU/g) as a function of irradiation dose (kGy). From this, a regression curve was obtained and the D_{10} value was calculated as the reciprocal of the absolute value of the slope of the curve (Microsoft Excel 5.0). The D_{10} value for a particular isolate was expressed as the average D_{10} value over 3

repetitions and the data was analyzed using the Statistical Analysis System software program General Linear Model (SAS Institute Inc., Cary, NC). Standard errors were calculated by using theTaylor series expansion.

2a. Assessment of 1.5 kGy surface dose for non-frozen comminuted meat (10^4 cells/g)

Ground beef

Fresh ground beef (15.0 - 19.5% fat) was purchased from 3 retail stores located in Ames, IA. the day before the experimental run. To obtain a uniform composite sample, equal amounts of ground beef were mixed with an Oster Food Grinder (Oster Corp., Milwaukee, WI), packaged in 100 g portions in Stomacher bags and stored at 4°C overnight. Composite samples were examined for indigenous *E. coli* O157:H7 as described below.

Sample preparation

A cocktail of the five most resistant *E. coli* O157:H7 strains was prepared by combining equal amounts of each individual stationary phase culture and diluting to a concentration of 10^6 cells/mL in 0.1% peptone water.

The pre-measured meat portions then were inoculated with 4 mL of the diluted cocktail (to give a concentration of 10^4 cells per gram) and blended for 2 min. with the Stomacher 400 Laboratory Blender. Samples (25 g) to be stored under air were packed into 60 mm by 15 mm petri dishes; lids were secured with Parafilm. Samples to be stored under vacuum were placed in a 6" x 8" vacuum packaging pouch (861 material grade, Weldon, Inc., Oshkosh, WI). Holes were punched into the lid of petri dishes, prior to filling with meat, for

air to be evacuated more effectively. Vacuum packaging and heat-sealing were done with a CVP Model A300 Fresh Vac modified air packaging machine (CVP Systems, Inc., Downers Grove, IL).

Irradiation and dosimetry

Samples were irradiated with the CICRE III Linear Electron Accelerator. All samples were subjected to a minimum surface dose of 1.5 kGy (110 kGy/min) using electron beam irradiation (10 MeV, 10 kW) at ambient temperature.

Absorbed doses were measured as described in objective 1.

Determination of irradiation survivors

After irradiation, samples were divided into 2 groups for storage at 4 and 12°C (refrigeration and abuse temperatures, respectively). Analysis was conducted on day 0, 5, 10, 20, and 30 for *E. coli* O157:H7 survivors. Twenty-five grams of meat were diluted 1:10 with non-modified TSB supplemented with cefixime (Lederle Labs, Pear River, NY) 0.05g/L, cefsulodin (Sigma Chemical Co., St. Louis, MO) 8 mg/L, and vancomycin (Sigma) 8 mg/L and blended with the Stomacher for 2 min. (see ref. 32). Diluted samples were incubated at 37°C for 6 hrs. After incubation, samples were stomached for 1 min., and subjected to immunomagnetic capture with subsequent spread-plating on sorbitol MacConkey agar (Unipath-Oxoid, Ogdensburg, NY) supplemented with cefixime and tellurite (CT-SMAC) as outlined by Dynal, Inc.(Lake Success, NY). Plates were aerobically incubated at 37°C overnight.

The next day, plates were examined for the presence/absence of typical *E. coli* O157:H7 colonies.   Typical colonies were further tested for positive latex agglutination (Unipath-Oxoid) and indole reactions (Remel, Lenexa, KY) and typical colony formation on EMB agar (Difco). The 30-day study was conducted in triplicate for samples irradiated and stored under air and in duplicate for samples irradiated and stored under vacuum. In addition, a smaller study of the same inoculum level was carried out in 2 repetitions. Samples were only irradiated under air and examined on day 0, 5, and 10 for survivors.

Identification of non-typical colonies

Non-typical colonies were picked off of the CT-SMAC plates and streaked onto XLD, Brilliant Green (BG) and EMB agars (Difco). Random colonies were chosen for further examination with the BBL Crystal Kit for Enterics (Becton Dickinson Microbiological Systems, Cockeysville, MD).

2b. Assessment of 1.5 kGy surface dose for non-frozen comminuted meat (10^3 cells/g)

The procedure described above was used for a smaller study involving an inoculation level of 10^5 cells/mL. Samples were examined on day 0, 5, and 10 for survivors. This experiment was conducted a total of five times.

RESULTS AND DISCUSSION

Bacteria of different genera differ in their sensitivity to irradiation; this is primarily attributed to their ability to repair DNA damage (36,55). The D_{10} values in Table 1 further

indicate that there is considerable variation in the radiation sensitivity of different strains within species ($p < .001$). Although a non-selective media was chosen for the enumeration of irradiation survivors, these D_{10} values may still underrepresent the radiation sensitivity of *E. coli* O157:H7. The ability of sublethally injured cells to recover from irradiation damage depends on optimizing the post-irradiation environment; media composition, incubation temperature and atmosphere influence cell repair (18).

The use of selective media for the enumeration of stressed cells has been cautioned. Components which may be normally non-inhibitory or non-toxic to an undamaged organism (i.e. salts and antibiotics) may adversely affect the resuscitation and repair of injured cells. Thus, viable and potentially prolific cells are excluded from survivor calculations. Clavero and Beuchat (12) found that non-selective TSA supported the highest recovery of heat- and freeze-stressed *E. coli* O157:H7 cells in ground beef, whereas modified sorbitol MacConkey agar was inferior. Tarté *et al.* (50) demonstrated that tryptic soy agar supplemented with 0.6% yeast extract was better suited to the recovery of electron-irradiated *Listeria* than modified Oxford medium.

X-ray irradiation was used in this study in order to deliver the low dose rate (kGy/min) required to achieve low doses. In practical terms, foods must be exposed to X-rays for a longer period of time to receive the same final dose than with accelerated electrons, which can deliver high dose rates (18). Although X-rays, like gamma rays (Co-60 or Cs-137), can penetrate deeper into material than electrons, this form of radiation is not economical for commercial applications as the efficiency of the conversion from electrons to X-rays is <10% (41).

Tarté et al. (51) compared the effects of X-rays and electron irradiation and found that both types were equally effective for the inactivation of a mixture of *Listeria monocytogenes* strains when treated at 4°C and -20°C. Irradiation type was a factor, however, at -78°C. D_{10} values were higher for electrons indicating that indirect effects played a greater role in bacteria inactivation.

Previous D_{10} values reported for *E. coli* O157:H7 include 0.27 kGy on TSA (52) for cells gamma-irradiated in beef under vacuum at 5°C and 0.241 to 0.307 kGy on SMAC (13) for refrigerated and frozen ground beef, respectively. The former investigators determined that log phase cells were more radiation-sensitive than stationary phase cells. This is not the case with all bacteria as Hastings et al. (30) found that *Lactobacillus sake* was more resistant in log phase than in stationary phase. Furthermore, age did not affect the D_{10} values of *Aeromonas hydrophila* (44). Thayer and Boyd (52) also noted that temperature influences D_{10} values. At -5°C, in mechanically deboned chicken meat, the D_{10} value for *E. coli* O157:H7 was 0.44 kGy, whereas at +5°C, the D_{10} value was 0.28 kGy. In general, as temperature decreases, D_{10} values increase due to the immobilization of free radicals derived from water; therefore, D_{10} values are higher in frozen food products (55).

Clavero et al. (13) determined that fat content (8-14% [low fat] and 27-28% [high fat]) did not affect the radiation resistance of this pathogen. Furthermore, *E. coli* O157:H7 in frozen hamburger patties (-17 to -15°C) were more radiation-resistant than in refrigerated (3 to 5°C) samples, thereby supporting the findings of Thayer and Boyd (52). In addition, it is important to mention that fat content or meat type (pork, lamb, beef, and poultry) does not

Table 1. D_{10} values for *E. coli* O157:H7 isolates[a]

Strain	D_{10} value	Standard error of D_{10}
NADC 3052	0.372	0.030
NADC 2922	0.280	0.020
NADC 2934	0.253	0.026
NADC 2903	0.244	0.010
ISU 052097[b]	0.235	0.010
NADC 3066	0.232	0.022
NADC 2952	0.213	0.027
NADC 2890	0.211	0.019
NADC 2893	0.208	0.032
NADC 2976	0.195	0.017
NADC 2924	0.191	0.003
NADC 2888	0.190	0.017
NADC 3061	0.185	0.008
NADC 3073	0.184	0.017
ATCC 43888[c]	0.177	0.020
NADC 2977	0.172	0.014
NADC 3076	0.170	0.014
NADC 3032	0.169	0.014
NADC 2918	0.167	0.024
NADC 2891	0.158	0.018
NADC 2881	0.154	0.006
NADC 2971	0.143	0.004
NADC 2962	0.141	0.008
NADC 2909	0.135	0.008
NADC 2892	0.132	0.019
NADC 3081	0.128	0.022
NADC 2939	0.087	0.006

[a] D_{10} values are expressed as the mean of three replicates
[b] Washington outbreak strain
[c] enterotoxigenic *E. coli*: does not produce SLT I or II

alter *E. coli* O157:H7 D_{10} values (52,53).

In the United States, the irradiation of poultry and pork is permitted for pathogen and parasite control, respectively (13,45). Currently, the FDA is reviewing a petition to allow the irradiation of fresh/chilled and frozen raw red meat for the elimination of pathogens (25).

Based on the highest D_{10} value obtained, it was theorized that at the minimum dose currently being reviewed by the FDA, 1.5 kGy for the irradiation of fresh/chilled raw red meat, *E. coli* O157:H7 may be eliminated at levels of 10^3 and 10^4 cells/g.

Sporadic survivors were recovered throughout the storage study (Tables 2-4). Electron irradiation at a minimum surface dose of 1.5 kGy, therefore cannot guarantee the complete inactivation of *E. coli* O157:H7 if present at levels of 10^3 and 10^4 cells/g. Data from the USDA, however, suggest that this pathogen is present in meat in low numbers, <15 organisms/g (26). Todd *et al*. (54) tested hamburger patties and beef implicated in 2 Canadian outbreaks. *E. coli* O157:H7 was detected at levels of 10 to 1000 cells/g.

Unlike gamma and X-ray irradiation, the maximum energy absorbance for accelerated electrons is not at the surface of the product. In fact, when an electron penetrates matter, a maximum dose is delivered below the product surface. In relation to our study, this means that up to a particular depth, the dose absorbed by the ground beef is greater than 1.5 kGy. This is due to the formation of secondary electrons in the food product. Because of their lower energy levels, these reactive species can be more effectively absorbed than the primary electrons. Eventually, a particular depth is reached where energy absorbance is at a maximum. As the primary electrons continue to travel through the product, their energy and velocity is diminished until they cannot penetrate any further. Electrons (10 MeV) can pass through a maximum of 3.3 cm (product density of 1g/cm^3) before their energy levels fall below that of the original surface dose. Penetration can be improved if the product is irradiated from both sides as doses in the low energy regions are additive (18,41).

Table 2. Number of samples positive for *E.coli* O157:H7 irradiated under air and vacuum (10^4 cells/g) [a,b,c,d]

Day	AIR						VACUUM			
	Rep 1		Rep 2		Rep 3		Rep 1		Rep 2	
	4°C	12°C	4°C	12°C	4°C	12°C	4°C	12°C	4°C	12°C
0	0	0	0	0	0	0	2	0	0	0
5	2	1	2	2	2	2	0	0	1	0
10	0	0	0	0	0	0	0	0	1	0
20	0	0	0	1	0	0	0	0	0	0
30	0	1	0	1	0	1	0	0	0	1

[a] untreated meat negative for indigenous *E. coli* O157:H7
[b] all control samples were positive for the presence of *E. coli* O157:H7
[c] two 25g samples examined per temperature per day
[d] positive samples : (+) indole, (+) latex agglutination, typical colony formation on EMB

Table 3. Number of samples positive for *E.coli* O157:H7 irradiated under air (10^4 cells/g) [a,b,c,d]

Day	Rep 1			Rep 2		
	4°C	12°C		4°C	12°C	
0	0	0		0	0	
5	0	1		0	1	
10	0	1		0	0	

[a] untreated meat negative for indigenous *E. coli* O157:H7
[b] all control samples were positive for the presence of *E. coli* O157:H7
[c] two 25g samples examined per temperature per day
[d] positive samples : (+) indole, (+) latex agglutination, typical colony formation on EMB

Table 4. Number of samples positive for *E.coli* O157:H7 irradiated under air (10^3 cells/g) [a,b,c,d]

Day	Rep 1		Rep 2		Rep 3		Rep 4		Rep 5	
	4°C	12°C	4°C	12°C	4°C	12°C	4°C	12°C	4°C	12°C
0	0	0	0	0	0	0	1	0	0	0
5	0	0	0	0	0	0	0	1	0	0
10	0	1	0	0	0	0	0	0	0	0

[a] untreated meat negative for indigenous *E. coli* O157:H7
[b] all control samples were positive for the presence of *E. coli* O157:H7
[c] two 25g samples examined per temperature per day
[d] positive samples : (+) indole, (+) latex agglutination, typical colony formation on EMB

Based on the initial inoculum levels and actual surface dose applied to the meat samples (data not shown), D_{10} values for surface survivors would theoretically be in the range of 0.38 to 0.51 kGy. These higher D_{10} values can be attributed to the exclusion of sub-lethally injured cells in the original calculation of the D_{10} values. As previously mentioned, sub-lethally injured cells, which are not detected immediately after irradiation, have the ability to repair DNA damage and later proliferate.

To determine if one strain was especially radiation-resistant, random typical colonies were picked off of CT-SMAC plates, streaked onto SMAC plates and then grown in luria broth for arbitrarily-primed PCR (data not shown; see ref. 34). Results revealed a mixed population of *E. coli* O157:H7 survivors.

On day 5, with reference to the samples irradiated and stored under air (Table 2), a "bloom" was observed; typical colonies of *E. coli* O157:H7 were isolated on CT-SMAC as well as other microflora. To see if this was related to storage under these conditions, one additional study was added (Table 3) and no unusual population bloom was observed. Furthermore, from the biochemical tests, bacteria from the *Enterobacter* spp. were dominantly isolated (these colonies did not cross-react with latex agglutination beads for the O157 antigen). To alleviate this interference, beads were washed three times before plating.

Previous studies have revealed that antiserum to O157 can cross-react with *Salmonella* group N, *Brucella* spp., *Yersinia enterocolitica*, and *E. hermanii* due to a common residue on their O157 antigen (10,11,43,46). Nevertheless, immunomagnetic

separation offers the advantage of concentrating target cells and reducing enrichment time prior to subsequent detection of more sensitive techniques such as PCR and ELISA (15).

Irradiation and storage under vacuum does not affect D_{10} values for *E. coli* O157:H7 (table 2); therefore, this data can be grouped together with the runs conducted under air. Thayer and Boyd (52) found that irradiation under air or vacuum did not affect D_{10} values for this organism. This was supported by Fu *et al.* (26) who irradiated and stored steak inoculated with 10^7 cells/g under air or vacuum for 9 days.

Although medium dose irradiation can increase the safety of meat products, it is not a fail-safe process. When added as the last step in a HACCP program and followed by the proper handling and cooking by consumers, *E. coli* O157:H7 elimination will then be assured. It would be of further interest to examine the irradiation processing parameters of frozen meat products as it is well known that organisms exhibit a greater radiation-resistance in frozen substrates (13,52). In addition, the sensitivity of other non-O157:H7 verotoxin-producing *Escherichia coli* implicated in outbreaks of hemorrhagic colitis and hemolytic uremic syndrome should be studied (4).

REFERENCES

1. ADA. 1996. Position of the American Dietetic Association: food irradiation. J. Am. Diet. Assoc. 96:69-72.

2. Barna, J. 1979. Compilation of bioassay data on the wholesomeness of irradiated food items. Acta Aliment. 8:205-315.

3. Barrett, T.J., J.B. Kaper, A.E. Jerse and I.K. Wachsmuth. 1992. Virulence factors in shiga-like toxin-producing *Escherichia coli* isolated from humans and cattle. J. Infect. Dis. 165:979-980.

4. Bettelheim, K.A. 1996. Enterohemorrhagic *Escherichia coli*: a new problem, an old group of organisms. Aust. Vet. J. 73:20-26.

5. Beutin, L., M.A. Montenegro, I. Orskov, F. Orskov, J. Prada, S. Zimmermann and R. Stephan. 1989. Close association of verotoxin (shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J. Clin. Microbiol. 27:2559-2564.

6. Brackett, R.E., Y.Y. Hao and M.P. Doyle. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. J. Food Prot. 57:198-203.

7. CAST. 1986. Ionizing energy in food processing and pest control: I. Wholesomeness of food treated with ionizing energy. Task Force Report No. 109. Council; for Agricultural Science and Technology, Ames, IA.

8. CDC. 1997. Outbreaks of *Escherichia coli* O157:H7 Infection and Cryptosporidiosis Associated with Drinking Unpasteurized Apple Cider--Connecticut and New York, October 1996. Morbid. Mortal. Weekly Rep. 46:4-8.

9. CDC. 1996. Outbreak of Escherichia coli O157:H7 infections associated with drinking unpasteurized commercial apple juice British Columbia, California, Colorado, and Washington, October 1996. Morbid. Mortal. Weekly Rep. 45:975.

10. Chart, H., O.A. Okubadejo and B. Rowe. 1992. The serological relationship between *Escherichia coli* O157 and *Yersinia enterocolitica* O9 using sera from patients with brucellosis. Epidemiol. Infect. 108:77-85.

11. Chart, H., T. Cheasty, D. Cope, R.J. Gross and B. Rowe. 1991. The serological relationship between *Yersinia enterocolitica* O9 and *Escherichia coli* O157 using sera from patients with yersiniosis and haemolytic uraemic syndrome. Epidemiol. Infect. 107:349-356.

12. Clavero, M.R.S., and L.R. Beuchat. 1995. Suitability of selective plating media for recovering heat- or freeze-stressed *Escherichia coli* O157:H7 from tryptic soy broth and ground beef. Appl. Environ. Microbiol. 61:3268-3273.

13. Clavero, M.R.S., J.D. Monk, L.R. Beuchat, M.P. Doyle, and R.E. Brackett. 1994. Inactivation of *Escherichia coli* O157:H7, salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. 60: 2069 - 2075.

14. Cray, Jr., W.C. 1994. (Unpublished data).

15. Cudjoe, K.S., P.D. Patel, E. Olsen, E. Skjerve and O. Olsvik. 1993. Immunomagnetic separation techniques for the detection of pathogenic bacteria in foods. In: New Techniques in Food and Beverage Microbiology.

16. D'Aoust, J.Y., C.E. Park, R.A. Szabo, E.C.D. Todd, D.B. Emmons and R.C. McKellar. 1988. Thermal inactivation of *Campylobacter* species, *Yersinia enterocolitica*, and hemorrhagic *Escherichia coli* O157:H7 in fluid milk. J. Dairy Sci. 71:3230-3236.

17. Dickson, J.S., and G.R. Siragusa. 1994. Survival of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during storage on beef sanitized with organic acids. J. Food Saf. 14:313-327.

18. Diehl, D.F. 1995. Safety of irradiated foods. 2nd ed. Marcel Dekker, Inc., New York, NY.

19. Dorsa, W.J., C.N. Cutter and G.R. Siragusa. 1997. Effects of steam-vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. J. Food Prot. 60:114-119.

20. Dorsa, W.J., C.N. Cutter, G.R. Siragusa and M. Koohmaraie. 1996. Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. J. Food Prot. 59:127-135.

21. Doyle, M.P., and J.L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. 48:855-856.

22. Doyle, M.P., and J.L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394-2396.

23. Engel, R.E., A.R. Post and R.C. Post. 1988. Implementation of irradiation of pork for Trichina control. Food Technol. 42:71-75.

24. Euro Surveillance. 1997. Scottish outbreak of *Escherichia coli O157:H7*, November - December 1996. http://www.b3e.jussieu.fr/ceses/EuroSurv/v2n1/en7-221.html.

25. FDA. 1994. Filing of food additive petition. Notice. U.S. Food and Drug Administration. Fed. Regist. 59:43848-43849.

26. Fu, A., J.G. Sebranek and E.A. Murano. 1995. Survival of *Listeria monocytogenes*, *Yersinia enterocolitica* and *Escherichia coli* O157:H7 and quality changes after irradiation of beef steaks and ground beef. J. Food Sci. 60:972-977.

27. Goldblith, S.A. 1966. Historical development of food irradiation, SM-73/54. Proc. Int. Symp. on Food Irradiation. International Atomic Energy Agency STI/PUB/127, Vienna, Austria.

28. Griffin, P.M., and R.V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60-98.

29. Hancock, D.D., T.E. Besser, M.L. Kinsel, P.I. Tarr, D.H. Rice and M.G. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect. 113:199-207.

30. Hastings, J.W., W.H. Holzapfel and J.G. Niemand. 1986. Radiation resistance of Lactobacilli isolated from radurized meat relative to growth and environment. Appl. Environ. Microbiol. 52:898-901.

31. IASR. 1996. Outbreaks of enterohemorrhagic *Escherichia coli* O157:H7 infection, 1996, Japan. http://www. nih.go.jp:80/yoken/iasr.

32. Jackson, T.C., M.D. Hardin and G.R. Acuff. 1996. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. J. Food Prot. 59:230-237.

33. Levine, M.M. 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Infect. Dis. 155:377-389.

34. Madico, G., N.S. Akopyants and D.E. Berg. 1995. Arbitrarily primed PCR DNA fingerprinting of *Escherichia coli* O157:H7 strains by using templates from boiled cultures. J. Clin. Microbiol. 33:1534-1536.

35. Martin, D.R., P.M. Uhler, A.J.G. Okrend and J.Y. Chiu. 1994. Test of Bob Calf fecal swabs for the presence of *Escherichia coli* O157:H7. J. Food Prot. 57:70-72.

36. Monk, J.D., L.R. Beuchat and M.P. Doyle. 1995. Irradiation inactivation of food-borne microorganisms. J. Food Prot. 58:197-208.

37. Morgan, D., C.P. Newman, D.N. Hutchinson, A.M. Walker, B.Rowe and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157:H7 infections associated with the consumption of yoghurt. Epidemiol. Infect. 111:181-187.

38. Morgan, G.M., C. Newman, S.R. Palmer, J.B. Allen, W. Shepherd, A.M. Rampling, R.E. Warren, R.J. Gross, S.M. Scotland and H.R. Smith. 1988. First recognized community outbreak of haemorrhagic colitis due to verotoxin-producing *Escherichia coli* O157 in the UK. Epidemiol. Infect. 101:83-91.

39. Mossell, D.A.A., J.E.L. Corry, C.B. Struijk and R.M. Baird. 1995. Essentials of the microbiology of foods. John Wiley & Sons, Inc., New York, NY.

40. Neill, M.A. 1994. *E. coli* O157:H7 time capsule: what do we know and when did we know it. Dairy Food Environ. Sanit. 14:374-377.

41. Olson, D.G. 1995. Irradiation processing, Ch.1. *In* E.A. Murano (ed.), Food irradiation: a sourcebook. Iowa State University Press, Ames, IA.

42. Padhye, N.V., and M.P. Doyle. 1992. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. J. Food Prot. 55:555-565.

43. Padhye, N.V., and M.P. Doyle. 1991. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl. Environ. Microbiol. 57:2693-2698.

44. Palumbo, S.A., R.K. Jenkins, R.L. Buchanan and D.W. Thayer. 1986. Determination of irradiation d-values for *Aeromonas hydrophila*. J. Food Prot. 49:189-191.

45. Pszczola, D. 1993. Irradiated poultry makes U.S. debut in Midwest and Florida markets. Food Technol. 46:89-92,94,96.

46. Rice, E.W., E.G. Sowers, C.H. Johnson, M.E. Dunnigan, N.A. Strockbine and S.C. Edberg. 1992. Serological cross-reactions between *Escherichia coli* O157 and other species of the genus *Escherichia*. J. Clin. Microbiol. 30:1315-1316.

47. Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Hebert, E.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake and M.L. Cohen. 1983.

Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New Engl. J. Med. 12:681-685.

48. Scotland, S.M., H.R. Smith and B. Rowe. 1985. Two distinct toxins active on vero cells from *Escherichia coli* O157. Lancet. ii:885-886.

49. Scotland, S.M., G.A. Willshaw, H.R. Smith and B. Rowe. 1987. Properties of strains of *Escherichia coli* belonging to serogroup O157 with special refernce to production of Vero cytotoxins VT 1 and VT 2. Epidemiol. Infect. 99:613-625.

50. Tarté, R., E.A. Murano and D.G. Olson. 1996. Survival and injury of *Listeria monocytogenes, Listeria innocua* and *Listeria ivanovii* in ground pork following electron beam irradiation. J. Food Prot. 59:596-600.

51. Tarté, R., D.G. Olson, E.A. Murano and P. Sundaram. 1996. Sensitivity of *Listeria monocytogenes* to irradiation in ground beef, as affected by type of radiation (x-rays or electrons), product temperature, packaging atmosphere, and recovery medium. (Paper accepted to the Journal of Food Science).

52. Thayer, D.W., and G. Boyd. 1993. Elimination of *Escherichia coli* O157:H7 in meats by gamma irradiation. Appl. Environ. Microbiol. 59:1030-1034.

53. Thayer, D.W., G. Boyd, J.B. Fox, Jr., L. Lakritz and J.W. Hampton. 1995. Variations in radiation sensitivity of foodborne pathogens associated with the suspending meat. J. Food Sci. 60:63-67.

54. Todd, E.C.D., R.A. Szabo, P. Peterkin, A.N. Sharpe, L. Parrington, D. Bundle, M.A.J. Gidney and M.B. Perry. 1988. Rapid hydrophobic grid membrane filter-enzyme-labeled antibody procedure for identification and enumeration of *Escherichia coli* O157 in foods. Appl. Environ. Microbiol. 54:2536-2540.

55. Urbain, W.M. 1986. Food Irradiation. Academic Press, Inc., Orlando, FL.

56. Weagant, S.D., J.L. Bryant and K.G. Jinneman. 1995. An improved rapid technique for isolation of *Escherichia coli* O157:H7 from foods. J. Food Prot. 58:7-12.

57. Wells, J.G., L.D. Shipman, K.D.Greene, E.G. Sowers, J.H. Green, D.N. Cameron, F.P. Downes, M.L. Martin, P.M. Griffin, S.M. Ostroff, M.E. Potter, R.V. Tauxe, and I.K. Wachsmuth. 1991. Isolation of *Escherichia coli* serotype O157:H7 and other shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. 29:985-989.

58. WHO. 1988. Food irradiation a technique for preserving and improving the safety of food. World Health Organization, Geneva, Switzerland.

59. Wilson, J.B., S.A. McEwen, R.C. Clarke, K.E. Leslie, R.A. Wilson, D. Waltner-Toews and C.L. Gyles. 1992. Distribution and characteristics of verocytotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. Epidemiol. Infect. 108: 423-439.

CHAPTER 5. GENERAL SUMMARY

In chapter 3, our study revealed that different strains of *Escherichia coli* O157:H7 differed in their resistance to X-ray irradiation. Subsequently, it was noted that electron beam irradiation at a surface dose of 1.5 kGy could not guarantee the complete inactivation of *E. coli* O157:H7 at levels of 10^3 or 10^4 cells/g in ground beef. However, it would be unlikely to find this organism in such high numbers. These findings stressed the importance of developing techniques that could improve the recovery of sub-lethally injured organisms, and thereby aid scientists in defining parameters for food processing methods.

It would be of interest to determine the irradiation D_{10} values of these strains under frozen conditions and examine the proposed minimum surface dose (~2.5 kGy) for frozen meat products. In addition, the effects of other atmospheres on the survival of *E. coli* O157:H7 should be studied. Finally, the irradiation sensitivity of non-O157:H7 verotoxin-producing *E. coli* warrants investigations.

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