

**Influence of dietary vitamin E on behavior of *Listeria monocytogenes* and color stability in ground turkey breast meat following electron beam irradiation**

by

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## CHAPTER 1. GENERAL INTRODUCTION

### INTRODUCTION

Contamination of turkey meat with *Listeria monocytogenes* represents a major concern to the food industry. One characteristic that makes this organism difficult to control is its ability to survive longer under adverse environmental conditions compared to other non-spore-forming foodborne pathogens. This characteristic, in addition to its ability to grow in a wide temperature range, from -1.5°C to 45°C, and in different atmosphere conditions makes *L. monocytogenes* a major food safety problem. Recent listeriosis outbreaks involving turkey meat products were associated with hundreds of reported cases, several deaths and costly product recalls in the United States. These foodborne outbreaks have directed much attention to the use of new technologies that could help in controlling and/or eliminating this pathogen in ready-to-eat or in cooked foods.

The technology of food irradiation can effectively eliminate *L. monocytogenes* and other vegetative foodborne pathogens in raw meat and poultry. Currently in the United States, legal approval of ionizing radiation to treat packaged poultry is limited to 1.5 to 3 kGy for the elimination of pathogens. Radiation inactivates microorganisms by damaging a critical element in the cell, most often the genetic material. This damage occurs as a result of a direct collision between the radiation energy and the DNA or via attack by reactive and diffusible free radicals from the radiolysis of water. Since water represents a large component of the cell, free radicals play a very important role in the destruction of microorganisms. Apart from the destruction of microorganisms radiation-induced free radicals are a major concern in

meats because of off-odor production and color changes, which reduce meat quality. Lipids and proteins become oxidized when exposed to irradiation, and the products created by oxidation are related to rancidity.

The meat industry has proposed the use of antioxidants to delay or prevent oxidation during meat irradiation. Vitamin E is a natural antioxidant which can be added endogenously to the meat through diet supplementation or exogenously by direct addition to ground meat. This antioxidant has the capability of scavenging free radicals, inhibiting lipid oxidation and improving the color of the poultry meat. However, a major safety concern is that the free radical-scavenging ability of antioxidants added to meat may reduce the antimicrobial effect of ionizing radiation. In general, previous published works have shown that supplementing diets of food animals with increased levels of vitamin E was not associated with changes in microbial growth during display of the meat. However, these studies did not use irradiation as a method to eliminate pathogens. In a relatively recent study, the addition of the antioxidant carnosine to ground turkey meat increased the radiation resistance of the pathogen *Aeromonas hydrophila*.

To our knowledge, no published reports on the combined effect of irradiation and dietary vitamin E supplementation on the radiation resistance and growth of *L. monocytogenes* in meat exist. Therefore, the objectives of this study were to evaluate the effects of dietary vitamin E supplementation on: 1) the survival of *L. monocytogenes* in vacuum-packaged raw ground turkey meat following irradiation, and 2) the growth of this microorganism during refrigerated storage (4°C) of aerobic- or vacuum-packaged irradiated



raw turkey meat. The effect of different levels of vitamin E in color stability of irradiated turkey meat in aerobic conditions was also determined.

### **THESIS ORGANIZATION**

This thesis is organized into a literature review (chapter 2), one paper (chapter 3) and general conclusions (chapter 4). The paper, entitled “Influence of dietary vitamin E on behavior of *Listeria monocytogenes* and color stability in ground turkey meat following electron beam irradiation”, has been submitted for publication in the Journal of Food Protection. It will be also presented at the International Association for Food Protection Annual Meeting in New Orleans on August 10-13, 2003. The reference citations throughout the thesis were formatted following the Journal of Food Protection Style Guide for Research Papers.

## CHAPTER 2. LITERATURE REVIEW

### *Listeria monocytogenes* and the genus *Listeria*

#### **Brief History**

*Listeria monocytogenes* was first described by Murray et al. in 1926 (64) after they isolated it from the blood of infected rabbits which had developed monocytosis, an abnormal increase in the number of monocytes (a type of white cell) in the circulating blood. They named the microorganism *Bacterium monocytogenes*. In 1927, Pirie also described a new microorganism during investigations of unusual deaths of rodents in South Africa and he called it *Listerella hepatolytica* (75). Once both strains were investigated, and were found to be the same microorganism, the isolated was renamed *Listerella monocytogenes*. One year later, Pirie proposed the name *Listeria* (76). It is believed that *L. monocytogenes* had been isolated several times prior to 1924, but its history starts in this year with the first confirmed diagnosis in a human that was suffering from meningitis, and the strain was isolated from cerebrospinal fluid (14).

#### **Taxonomy of the genus *Listeria***

Based on evidence collected by DNA homology values, 16S rRNA sequencing homology, chemotaxonomic properties, and multilocus enzyme analysis, the genus *Listeria* currently contains six species: *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri*, and *Listeria grayi* (79).

## **Subtyping *Listeria monocytogenes***

### **Serotyping**

Serotyping has poor discriminating power when compared with other subtyping methods. Nevertheless, it provides valuable information for rapid screening of strains isolated during suspected outbreaks. It allows elimination of isolates that are not part of an outbreak and facilitates efficient application of other more sensitive but time-consuming subtyping methods (36). Strains of *L. monocytogenes* differ in the antigenic determinants expressed on the cell surface. This difference can be identified by serological typing. The serotyping scheme for members of the genus *Listeria* is based on 5 heat-labile flagellar (H) antigens and 14 heat-stable somatic (O) antigens (84). Most of the human infections (>95%) are caused by strains of *L. monocytogenes* that belong to the serotypes 1/2a, 1/2b, and 4b (2).

### **Other typing methodologies**

*L. monocytogenes* has been also characterized using bacteriophage typing, bacteriocin typing, and molecular methods such as multilocus enzyme electrophoresis, chromosomal DNA restriction endonuclease analysis (REA), restriction fragment length polymorphism analysis (ribotyping), and DNA macrorestriction analysis by pulsed-field gel electrophoresis (PFGE). In addition, random amplification of polymorphic DNA (RAPD), repetitive element-based sub-typing, and DNA sequence-based subtyping have been used (36). From all these identification methods, serotyping, phage typing, REA, PFGE, and RAPD were selected for standardization by the World Health Organization Multicenter *Listeria monocytogenes* Subtyping Study (2).

## Identification of bacteria of the genus *Listeria*

*Listeriae* are gram-positive, regular, small rods (0.5 µm in diameter and 1-2 µm in length), with rounded ends. This genus does not produce spores and capsules are not formed. *Listeria* is motile with peritrichous flagella which produces when cultured at 20-25°C. This microorganism is aerobic, microaerophilic, facultatively anaerobic, catalase-positive, and oxidase-negative. All strains grow on glucose forming lactate, acetate, and acetoin. Also, they are methyl-red and Voges-Proskauer test positive. *Listeriae* do not produce acid from adonitol, arabinose, dulcitol, erythritol, glycogen, inositol, inulin, melibiose, raffinose, or sorbose. They do not hydrolyze urea. In addition, indole, H<sub>2</sub>S, phenylalanine-deaminase, ornithine, lysine, and arginine decarboxylases are not produced (79).

## Characteristics of *L. monocytogenes*

### Temperature

*L. monocytogenes* has common features of both psychrotrophic and mesophilic bacterium, thus this microorganism grows in a very wide temperature range. The optimal growth temperature for *L. monocytogenes* ranges between 30 to 37°C (74). Walker et al. (98) confirmed the ability of this pathogen to multiply during extended incubation at temperatures as low as -0.1 to -0.4°C in chicken broth and pasteurized milk. The lowest growth temperature for *L. monocytogenes* was reported by Hudson et al. (39), who demonstrated that the organism grew at -1.5°C in vacuum-packaged sliced roast beef. Although this pathogen does not grow below -1.5°C, it can readily survive at much lower temperatures. Palumbo et al. (71) reported the ability of *L. monocytogenes* to survive freezing and frozen storage at -

18°C in ground turkey. The viable count of this pathogen remained essentially constant when the organism was suspended in ground meat after 14 weeks of storage at -18°C. El-Kest et al. (22) exposed *L. monocytogenes* to freezing temperatures as low as -198°C. This temperature was minimally detrimental to cells.

### **Acidity**

*L. monocytogenes* normally grows at pH values from 5.6 to 9.6, with optimal growth at neutral to slightly alkaline pH values (74). Although growth of *L. monocytogenes* at pH <4.3 has not yet been reported, this organism appears to be fairly acid-tolerant. Reimer et al. (77) recovered this pathogen from inoculated samples of citrate/phosphate buffer that were acidified to pH 3.3 and held for 4 h at 37°C.

### **Salt Tolerance**

*L. monocytogenes* is a salt tolerant microorganism. It can grow slowly in nutrient broth supplemented with up to 10 to 12% (w/v) NaCl (85). The salt tolerance has been related to the incubation temperature, because the survival of *L. monocytogenes* in concentrated salt solutions can be significantly increased by lowering the incubation temperature (39).

## **Listeriosis in Humans**

### **Asymptomatic Carriage**

*L. monocytogenes* is distributed throughout the environment and can be frequently recovered from a broad spectrum of foods, and from the gastrointestinal tract of healthy people (87). Numerous surveys have reported the prevalence of this microorganism in humans. Thus, 4.8% of 1,147 healthy slaughterhouse workers had stool cultures yielding the organism as well as 1.2% of 1,034 hospitalized adults with diarrhea (3). Among pregnant women, 2% of 51 women in early pregnancy were fecal carriers of this microorganism. This incidence was very similar to the 3.4% among 59 non-pregnant women attending the same clinic (47).

### **Noninvasive Disease**

It has been postulated that a noninvasive gastrointestinal illness may occur in normal hosts that consume foods contaminated with an infectious dose of *L. monocytogenes* (87). When *L. monocytogenes* was isolated from contaminated chocolate milk, which was related to an outbreak, 79% of 58 people who consumed the product had diarrhea and 72% reported fever (15). The frequency of febrile gastroenteritis caused by this pathogen remains undetermined, as does the infectious dose and characteristics of the host that are associated with this disease (87).

**Invasive disease in non-pregnant adults**

Listeriosis in non-pregnant adults is associated with some clinical conditions that compromise the immune system. Among these, organ transplants, immunosuppressive therapy, infection with the human immunodeficiency virus (HIV), and advanced age can be mentioned (83). The clinical syndromes in non-pregnant adults with listeriosis are sepsis, meningitis, meningoencephalitis, and bacteremia (31). In addition to these syndromes, endocarditis, focal infections, and infections in other parts of the body may occur (29). Some of the symptoms are fever, malaise, ataxia, seizures, and altered mental status (87).

**Listeriosis during pregnancy**

Listeriosis may occur at anytime during pregnancy but is most frequently reported during the last trimester. The symptoms associated with listeriosis during pregnancy are non-specific. Usually the only manifestation is a mild flu-like illness, with fever, headache, myalgia, or gastrointestinal symptoms (82). Intrauterine infection is believed to result from transplacental transmission of the pathogen following maternal bacteremia. As a result of this infection, preterm labor, amnionitis, spontaneous abortion, stillbirth, or early-onset neonatal infection may occur. Perinatal listeriosis is rarely life-threatening for the mother (30).

**Neonatal Listeriosis: Early onset**

Early-onset neonatal listeriosis occurs in infants that have been infected in utero. It results in illness at birth or shortly (hours or a few days) thereafter. The disease often

involves sepsis and less frequently, granulomatosis infantiseptica (a chronic condition marked by the formation of numerous nodules of chronically inflamed tissue that is usually associated with an infective process) (31). The prognosis for infants in the early onset is usually very poor. A fatality rate of 38% has been reported (50).

#### **Neonatal Listeriosis: Late onset**

The transmission of *L. monocytogenes* in the late onset is not very clear. It has been postulated that transplacental transmission occurs, but it may also be acquired during passage through the birth canal. Listeriosis may occur from one to several weeks after birth, and meningitis is the most frequent syndrome during late-onset of the disease (31). A fatality rate of 25% has been reported (50).

#### **Epidemic foodborne listeriosis**

The first evidence of foodborne transmission of listeriosis was documented in 1981 from an outbreak in the Maritime Provinces of Canada (81). During a six month period, 34 perinatal cases (including 5 abortions, 4 stillbirths, and 23 infants born seriously ill) and 7 adult cases were reported. The isolated epidemic strain was *L. monocytogenes* 4b. Coleslaw was the implicated vehicle of transmission. The cabbage used in the coleslaw came from a farm where cases of listeriosis in sheep had occurred, and the cabbage field had been fertilized with raw sheep manure.

In Massachusetts, 1983, a large outbreak was identified, where 49 cases occurred in a period of 2 months (42 immunosuppressed adults and 7 pregnant women) (28). Pasteurized



milk was documented as the vehicle of infection. Mexican-style soft cheese has been involved in large outbreaks of listeriosis in North America (49). Pregnant women accounted for 93 cases, and non-pregnant adults did for 49 cases (47 had predisposing conditions for listeriosis). The case fatality rate was 32% among the perinatal cases, and 37% in the non-pregnant adults. More outbreaks have been related to meat pâté in 1987-1989 (51) and pork pâté in 1992 (35).

In May 2000, 29 illnesses caused by a strain of *L. monocytogenes* were identified in 10 states of North America (11). When subtyped, the *L. monocytogenes* isolates from these cases were indistinguishable by PFGE ribotyping. The outbreak was linked to deli turkey meat. Eight perinatal and 21 non-perinatal cases were reported. The 29 cases were associated with four deaths and three miscarriages/stillbirths. State health and agriculture departments investigated 13 stores and delicatessens where 11 patients reported purchasing turkey; these stores and delicatessens carried turkey meat produced by at least 27 federally inspected establishments. Two of the establishments were linked to 10 of 11 patients. In December 2000, Cargill Turkey Products, Inc. (Waco, Texas) stopped shipping ready-to-eat foods and, and voluntarily recalled processed turkey and chicken deli meat that might have been contaminated.

In November 2000, twelve cases of listeriosis within a 2-month period in North Carolina were reported (10). The outbreak implicated non-commercial, homemade, Mexican-style fresh soft cheese produced from contaminated raw milk sold by a local dairy farm as the causative agent. *L. monocytogenes* isolated from patients, raw milk, and cheese were tested using PFGE. Environmental inspections of homes, local markets, and dairy farms

were conducted. As a result of this outbreak, North Carolina health authorities stopped the sale of raw milk by the dairy farm to noncommercial processors and educated store owners that it is illegal to sell unregulated dairy products.

The last outbreak that has been reported in North America was in September 2002 (9). A multistate outbreak of *L. monocytogenes* infections with 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages in eight states was reported. It was linked to eating sliceable turkey deli meat. Outbreak isolates shared a relatively uncommon PFGE pattern. One intact food product and 25 environmental samples from a poultry processing plant yielded *L. monocytogenes*. The isolate from the food product had a PFGE pattern different from the outbreak strain. However, two environmental isolates from floor drains shared a PFGE pattern indistinguishable from that of outbreak patient isolates, suggesting that the plant might be the source of the outbreak.

### ***Listeria monocytogenes* in the environment and in poultry meat**

#### ***L. monocytogenes* in the environment**

*L. monocytogenes* is considered an ubiquitous microorganism. It can survive longer under adverse environmental conditions than many other non-spore forming bacteria of importance in foodborne disease (27). It has been isolated from soil, which is often referred as the source of *Listeria*, since soil receives decaying plant material, animal waste, sewage sludge, all of which are sources of *L. monocytogenes* (99). Fecal material from a wide variety of animal species has been found to carry *L. monocytogenes*. Among these animals, sheep, goats, cattle, pigs, chickens, and turkeys can harbor the pathogen (65, 96).

### ***Listeria monocytogenes* in poultry meat**

*L. monocytogenes* has been found in raw as well as processed poultry products. In one survey *Listeria* was isolated from 45% of fresh turkey wings, 28.3% of fresh legs, and 23.3% of fresh tails (32). In another survey 9 of 42 turkey carcasses tested positive for *Listeria* following defeathering (13). This survey demonstrated that fresh turkey parts are likely to become contaminated with *Listeria* during later stages of processing (evisceration, chilling). Ryser et al (80) reported that 33 of 45 (73%) retail samples of ground turkey contained *Listeria* spp., including a diverse group of *L. monocytogenes*. In 2002, a multistate outbreak of *L. monocytogenes* infections with 46 culture-confirmed cases was linked to eating sliceable turkey deli meat. One intact food product and 25 environmental samples from a poultry processing plant yielded *L. monocytogenes*. On the basis of these findings, the plant recalled 27.4 million pounds of fresh and frozen ready-to-eat turkey and chicken products (9).

### **Transmission of *Listeria monocytogenes***

The natural environment appears to be the initial reservoir for virulent strains of *L. monocytogenes* (Fig. 1). Silage that contain this pathogen, when stored in silos, allow its multiplication. When ruminants are fed this silage they become infected and may contaminate soil, equipment, and environmental sources through *L. monocytogenes*-fecal contamination. Equipment and environmental sources may also become contaminated through sewage, polluted water, and humans (27).

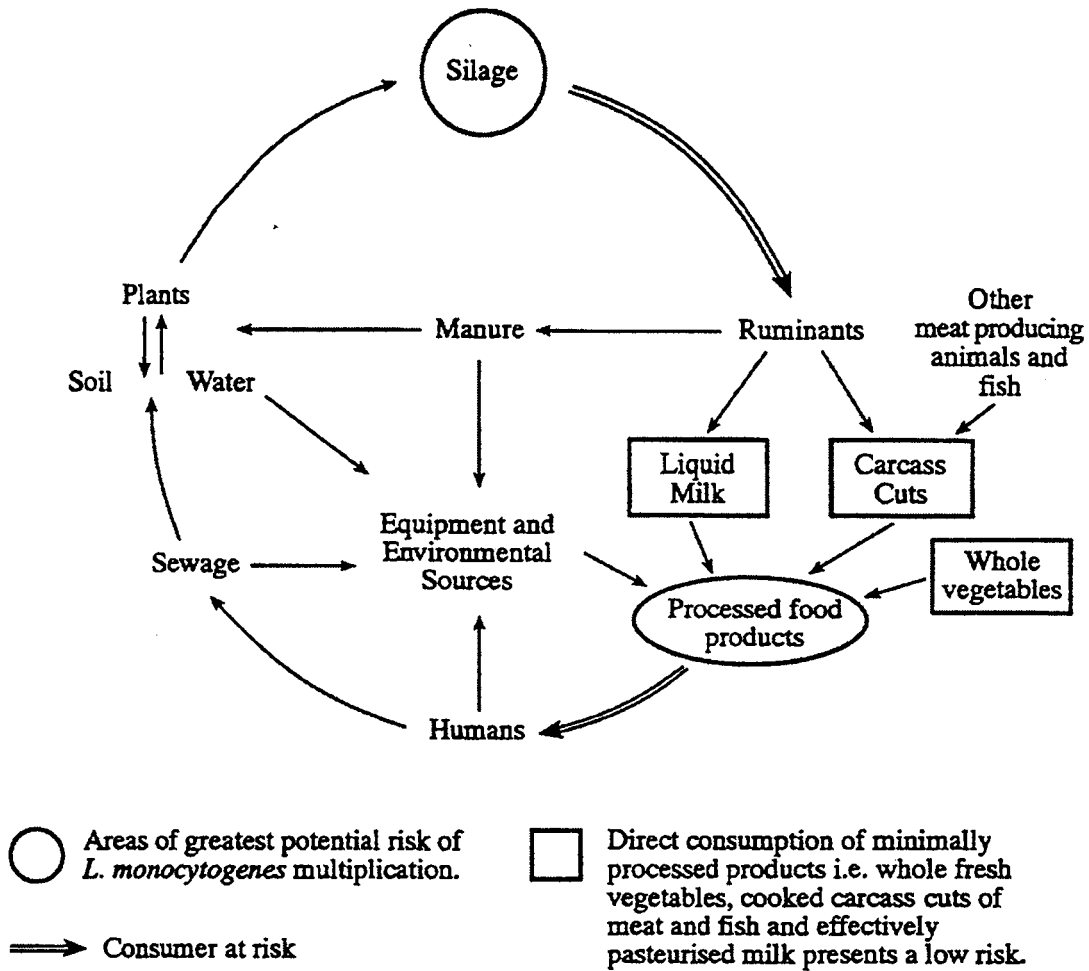


Fig. 1— Spread of *Listeria monocytogenes* to the food chain from the natural environment (From Ref. 27)

Processed food products become contaminated through *Listeria* on food processing equipment or the processing plant environment, and also when the product comes from animals that were previously infected (liquid milk, carcass cuts). Humans become infected through consumption of contaminated products, and the cycle starts again when humans contaminate sewage water that goes directly to the soil (27).

## **Food Irradiation**

### **Brief history and current status of Food Irradiation**

Food irradiation had its beginnings in the last years of the 19<sup>th</sup> century, when von Roentgen discovered X-rays in 1895 and Becquerel observed radiation from uranium in 1896. In 1905 Appleby and Banks suggested the use of ionizing radiation to kill microorganisms in food, especially cereals, with alpha, beta or gamma rays from radium or other radioactive substances. In 1921, Schwartz obtained a U.S. patent on using X-rays to kill *Trichinella spiralis* in meat. A French patent was granted in 1930 to Wüst “to preserve foods of all kinds which are sealed packed in sealed metallic containers and submitted to the action of hard Roentgen rays of high tension to kill bacteria”. It was not until after the World War II, with the advent of nuclear age, that food irradiation received serious consideration. During this time the capability was acquired to produce enough curies of <sup>60</sup>Co and <sup>137</sup>Cs at a reasonable price and suitable machine sources of ionizing energy were developed (20, 44).

### **Food irradiation in the United States**

After World War II, food irradiation studies began at the Massachusetts Institute of

Technology, at Electronized Chemicals, Inc., and at the General Electric Company Research Laboratories. The Atomic Energy Commission (AEC) in 1950 and the Army Quartermaster Corps in 1953 assumed the role as U.S. government sponsors of food irradiation programs. In 1953, President Eisenhower proposed the “Atoms for Peace” program, which led to the establishment of a National Food Irradiation Program and the formation, in 1956, of the Interdepartmental Committee on Radiation Preservation of Foods. Most of the work on the National Food Irradiation Program was conducted by the Army, which was later joined by the AEC. The U.S Army supported research on both low- and high-dose applications. In 1958, the Food and Drug Administration (FDA) defined ionizing radiation as a new food additive (20, 44).

After 1960, the Army concentrated its support on high-dose applications, specifically on the development of radiation-sterilized meat products to substitute for canned or frozen military rations. In 1966, the Army submitted a petition to the FDA and USDA to approve radappertized ham. In the absence of long-term toxicity studies for ham the FDA ruled that the data were insufficient and it did not approve the petition. Similar actions were taken with bacon and pork. These events caused a very significant slowdown of food irradiation activity in U.S. In 1980, the Army’s contracts to assess the wholesomeness of radappertized beef, pork and ham were terminated, and the government’s food irradiation program was transferred to the U.S. Agriculture’s Eastern Regional Research Center in Philadelphia, PA (20, 44).

It was not until mid 1980s when a renewed interest in the commercial prospects of food irradiation developed. Since that time, the FDA has approved irradiation of dry

spices/seasonings at a maximum dose of 30 kGy for microbial disinfection, dry enzymes preparation at a maximum dose of 10 kGy for microbial disinfection, fresh pork at a minimum dose of 0.3 kGy and a maximum of 1 kGy for *Trichinella spiralis* control, and certain fresh foods at a maximum dose of 1 kGy for growth and maturation inhibition. FDA approval was also given for arthropod disinfection of some agricultural products at a maximum dose of 1 kGy, poultry at a maximum dose of 3 kGy for pathogen control, frozen meats for NASA consumption at a maximum dose of 44 kGy for sterilization, refrigerated red meat at a maximum dose of 4.5 kGy for pathogen control, and frozen meat at a maximum dose of 7 kGy for pathogen control (58). Early in 1993, the United States' first pilot plant food irradiator designed specifically for the irradiation of meat and poultry, an electron beam/X-ray facility, started operating at Iowa State University in Ames, IA.

### **Units to measure irradiation**

The irradiation dose applied to a food is the most important factor of the irradiation process. The unit of absorbed dose used in the past is called a rad. A rad is equivalent to 100 ergs of energy absorbed per gram of irradiated material. The currently used unit of absorbed dose is the Gray (Gy), which is equivalent to 100 rads or the absorption of 1 joule of energy per kilogram of irradiated material. One kilogray (kGy) is equivalent to 100,000 rads (53).

### **Forms of irradiation**

The electromagnetic spectrum is composed of at least six separate forms of radiation that differ in wavelength, frequency, and penetrating power (53). Of these forms, gamma

radiation, ultraviolet (UV) radiation, and microwaves are of interest to the food industry. The two most widely used techniques are gamma irradiation from either  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  and the use of electron beams from linear accelerators.

### **Gamma irradiation**

Gamma rays have approximately 1-2 million electron volts (MeV) of energy and can penetrate materials with a thickness of about 40 cm. These rays are emitted by radioisotopes such as  $^{60}\text{Co}$  and  $^{137}\text{Cs}$  (53).  $^{60}\text{Co}$  is produced in a nuclear reactor via neutron bombardment of highly refined  $^{59}\text{Co}$  pellets, whereas  $^{137}\text{Cs}$  is produced as a result of uranium fission and may be reclaimed as a byproduct of nuclear fuel reprocessing.  $^{60}\text{Co}$  is the radioisotope of choice for gamma irradiation of food as sources are readily available. The technology for production, fabrication and encapsulation is highly developed and  $^{60}\text{Co}$  has greater penetration power than does  $^{137}\text{Cs}$  (91).

### **Electron beams/Accelerated electrons**

Powered by electricity, electron-beam machines use linear accelerators (“LINACs”) to produce high voltages of electron beams accelerated to very high speed. Electron beams are highly efficient since they can be focused directly onto food products to allow high plant-product capacity. Also, they can be switched on and off like a light bulb, and are not related to the nuclear industry (42, 91). The ability to program and to regulate automatically from one instant to the next with simple electronic detectors and various beam parameters shows how the use of this technology is very suitable for efficiently processing foods (42).



However, unlike gamma irradiation, high-energy electron beams have limited penetration power and are suitable only for foods and materials of relative shallow depth (5-10 cm) (42, 53, 91).

Electron beam accelerators are highly complex machines. An electron accelerator consists of a hot filament that emits electrons into an evacuated chamber. A high positive electric potential then attracts these electrons and focuses them into a narrow beam. The final step involves the passage of the beam through the poles of an electromagnet with a constantly changing magnetic field that causes the electron beam to sweep from side to side. This sweeping beam is then directed to the food (33). Electron beams can also be converted into X-rays via their bombardment of heavy metals such as tungsten (53). X-rays have a high-penetration power; however, this is an expensive technique (91).

### **Application of Radiation**

Foods are treated with ionizing radiation to accomplish many different goals. This food processing technology can improve the safety of food through the reduction of pathogenic bacteria and other microorganisms and parasites that cause foodborne diseases. Irradiation also inactivates food spoilage organisms, including bacteria, molds, and yeasts. It can be effective in lengthening the shelf-life of fresh fruits and vegetables by decreasing the normal biological changes associated with growth and maturation processes, such as ripening or sprouting (58).

### **Control of microorganisms**

The application of irradiation to food in order to control microorganisms may be categorized as low, medium, and high dose, and may be defined as radurization, radicidation, and radappertization.

Radurization: This is the treatment of food with a dose of ionizing radiation sufficient to enhance its quality by reducing the numbers of viable specific spoilage microorganisms (95). It is called radiation pasteurization, and involves use of doses that range from 0.75 to 2.5 kGy to reduce populations of viable spoilage microorganisms in foods such as fresh meats, poultry, seafood, vegetables, fruits, and cereal grains. However, the effectiveness of this method for improving the safety and shelf-life of foods can be limited by the survival of mostly psychrotrophic gram-positive bacteria and some gram-negative bacteria of the genera *Moraxella* and *Acinetobacter* (42, 53).

Radicidation: This is the treatment of food with a dose of ionizing irradiation sufficient to reduce numbers of viable specific non-spore-forming pathogenic bacteria to such a level that none is detectable in the treated food when it is examined by any recognized bacteriological testing method (95). Radicidation involves irradiating foods to destroy vegetative foodborne pathogens. Typical doses range from 2.5 to 10 kGy. Viruses and spores of pathogens are not destroyed, and some radiation-resistant strains of bacteria can survive (53).

Radappertization: This is defined as the treatment of food with a dose of ionizing radiation sufficient to reduce the number and/or activity of viable microorganisms to such a level that a very few, if any, are detectable by any recognized bacteriological or mycological

testing method applied to the treated food. It does not include inactivation of viruses or bacterial toxins (95). Radappertization involves the application of high irradiation doses (30-40 kGy) for destruction of *Clostridium botulinum* spores (53, 95).

### **Inhibition of sprouting and delay of ripening and senescence**

This use of irradiation is limited to fresh fruits and vegetables. Although the irradiation treatment is not specifically aimed at controlling foodborne microorganisms, inhibition of sprouting in food such as onions and potatoes and the delay of ripening in other fresh vegetables and fruits extend the shelf-life of these products. Doses fall in the range of about 0.02 to 3 kGy (95).

### **Decontamination**

This use of irradiation has as its objective the reduction of a microbial population, but not for the purpose of preservation of the food. Instead, it is used to reduce the microbial population of food ingredients such as spices, seasonings, starches, and gums. This is important because these ingredients are known sources of microbial contamination of foods. Doses range from 3 to 30 kGy (95).

### **Insect disinfestations**

Insects in foods are undesirable because of the damage caused to foods, the consumer objection to their presence in foods, and the spreading of insects from one location to

another. The eggs of insects and insect larvae can be destroyed by 1 kGy. Doses applied for insect disinfestations range from 0.1 to 1 kGy (95).

### **Quality improvement**

In most cases, when foods are irradiated, one of two kinds of chemical changes is involved: 1) depolymerization of large molecules such as carbohydrates or proteins, and 2) cell injury with consequent easier release of cell content (95). Some of the resulting improvements may include: (a) an increase in the rates of hydration of dehydrated vegetables (e.g., soup mixes), (b) an increase in the yield of juice from grapes, (c) an increase in the drying rate of fruits (e.g., prunes), (d) a reduction in the cooking time of certain foods (e.g. dried beans), (e) an increase in the volume of loaves of bread, (f) a reduction in the flatulence-causing ability of beans, (g) a reduction in the amount of sodium nitrite required in cured meats, and (g) meat tenderization (8). Doses needed to achieve this effect are likely to fall in the range of 0.1 to 10 kGy, depending on the quality improvements sought (95).

### **Mechanisms of inactivation**

Radiation inactivates microorganisms by damaging a critical element in the cell, most often the genetic material. This damage prevents multiplication and also randomly terminates most cell functions. Ionizing radiation interacts with an irradiated material by transferring energy to electrons which are thus raised to a higher-energy, or excited state (61). If the transferred energy is large enough, the negatively charged electron can leave a molecule and a positive ion is formed. The ejected electron moves through surrounding material and

loses energy by creating further excited molecules and positive ions. Eventually the electron is captured by a positive ion or is trapped by a structure to form a negative ion which in turn combines with a positive one. It is this ability to create positive and negative ions that characterizes ionizing radiation (61).

Damage of the genetic material occurs as a result of a direct collision between the radiation energy and the genetic material, or as a result of radiation ionizing an adjacent molecule, generally water, which in turn reacts with the genetic material (18, 61). In the first case the effects are direct, and in the second case the effects are called indirect.

### **Direct Action**

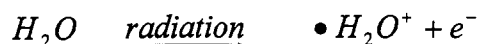
This term is used to describe the chemical events occurring in the target molecule, DNA, as a result of energy deposition by radiation in this molecule. In the majority of bacteria there is only one copy of the chromosome, which exists as an extremely long (about 1 mm) doubled-stranded circular molecule. Ionizing radiation is non-discriminating, therefore, all components of the cell absorb radiation and are damaged in proportion to their mass, with those of higher mass enduring relatively more damage. Thus, since ionizing radiation may deposit energy in any of the atoms that make up DNA, the number of alterations that can occur in DNA runs into hundreds (61). During the direct effect, a photon of energy or an electron randomly strikes the genetic material of the cell and causes a lesion in the DNA. The lesion can be a break in a single strand of the DNA or, if the orientation of this molecule is appropriate, the energy or electron can break both strands.

Single-strand lesions may not be lethal and may in fact result in mutations. However, large numbers of single-strand lesions may exceed the bacterium's DNA repair capability, which ultimately results in the death of the cell. A double-strand lesion occurs when the photon or electron strikes adjacent areas on both strands of the DNA. This severs the molecule into two pieces and usually results in lethality because such damage is more difficult to repair. However, because of the necessary orientation of the DNA in relation to the irradiation source, double-strand lesions occur much less frequently than single-strand lesions (18). Generally, the number of double strand breaks is 10- to 20-fold less than the number of single strand breaks (62).

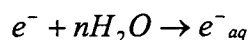
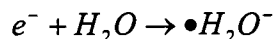
### **Indirect Action**

Since the major component of a cell is water, the majority of the ionizing events will occur in water. The indirect effect occurs as a consequence of reactive, diffusible free radicals (formed from the radiolysis of water) reacting with the DNA. The radiolysis of water results in the formation of a number of reactive species such as the hydroxyl radical ( $\text{OH}^\bullet$ ), the hydrated electron ( $e^-_{\text{aq}}$ ), the  $\bullet\text{H}$  radical, and hydrogen peroxide. All these species are reactive, but the major effective specie is the  $\text{OH}^\bullet$  radical which reacts with organic molecules either by adding to a double bond or by extracting an H atom from a C-H bond to form water and a carbon radical (61).

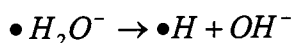
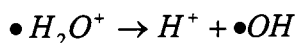
When pure water is irradiated it is ionized, yielding a free electron and a positively charged water ion:



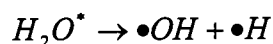
This electron can then react with another water molecule to yield a negatively charged water ion, or it can become hydrated:



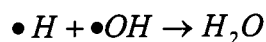
The hydrated, or aqueous, electron ( $e^{-}_{aq}$ ) is formed when an electron, after having lost most of its kinetic energy by collisions, attracts several molecules and becomes surrounded by them, with the electric dipole moments of the water molecules oriented towards the electron's charge. Hydrated electrons have impaired mobility and are most stable than free electrons. Nevertheless, they are still very reactive and behave very much like  $\bullet H$  radicals (70). Neither of the two water ions produced are very stable, so they both dissociate into an ion and a free radical:



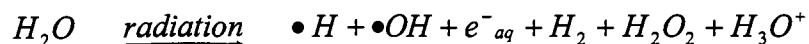
Some excited water molecules can also yield free radicals:



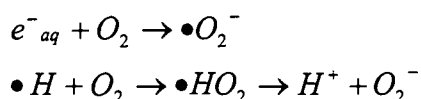
While some  $\bullet H$ ,  $\bullet OH$ , and  $e^{-}_{aq}$  radicals can react with solute molecules, they can also recombine with each other:



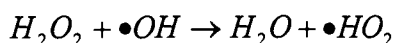
All these reactions can be pooled together and their products shown in a more simplified equation for the irradiation of pure water (70, 95):



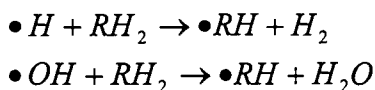
Molecular oxygen can react with  $e^-_{aq}$  and  $\bullet H$  radicals (70):



Where  $\bullet O_2^-$  is superoxide anion and  $\bullet HO_2$  is hydroperoxide radical. The free radicals from water can also react with their own reaction products to yield free radicals:



or they can also react with surrounding organic molecules to yield secondary free radicals by hydrogen abstraction (70):

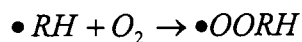


where RH represents an organic molecule, such as a protein, lipid, or carbohydrate.

Secondary radicals are those formed by the reaction of a primary radical from water and a molecule that is not a radical (8). It must be pointed out, however, that similar free radicals can also be formed by direct action of radiation:



These organic free radicals, either primary or secondary, can react with molecular oxygen to yield a variety of peroxide radicals (8):



These peroxide radicals can then react with hydrogen to form hydroperoxides (HOORH)

which can, in turn, yield oxidized products. Therefore, these free radical reactions that occur in the presence of oxygen are very significant from the stand point of food quality, especially



in meat products, since their products will usually contribute to rancidity (92). Free radicals finally become stabilized by either reacting with each other or with solute molecules.

Free radicals react with the nucleic acids and the chemical bonds that bind one nucleic acid to another in a single strand, as well as with the bonds that link the adjacent base pair in the opposite strand. As with the direct action of irradiation, the indirect action can result in single- and double-strand lesions, with the same overall effects (18).

### **Nature of radiation injury**

#### **DNA strand breakage**

Of the lesions produced in DNA by ionizing radiation, the one most closely related with cell killing is the breakage of one or both strands on the deoxyribose moiety of the sugar-phosphate backbone of DNA. About 20% of the hydroxyl radical reactions with DNA result in the removal of hydrogen atoms from any of the five carbon atoms of the deoxyribose moiety and subsequent  $\beta$ -elimination leads to strand breaks usually via cleavage of the C-3' phosphate ester bond (62).

The introduction of strand breaks into bacterial DNA by ionizing radiation can be followed by measuring the sedimentation velocity of irradiated DNA and comparing it with that of DNA from non-irradiated bacteria. As the DNA accumulates breaks, the average molecular weight of DNA is reduced and this is reflected by a slower rate of sedimentation. In order to perform this method, high molecular weight single-strand DNA from bacteria has to be isolated by lysing cells in an alkaline 5 to 20% sucrose gradient. A DNA double-strand break is formed when the two strands of the double helix are broken opposite each other or at

least sufficiently close to each other that the hydrogen-bonding between the two sites is insufficient to maintain the structure.

Double-strand breaks can be detected by measuring the sedimentation velocity of DNA from irradiated bacteria in neutral, rather than alkaline, sucrose gradients and comparing it with that of DNA from non-irradiated bacteria (61).

### **Base damage**

Most of damage to DNA irradiated in cells is caused by the hydroxyl radical, a radiolytic product of water. About 20% of this attack is on DNA sugars and nearly 80% on bases, with the sensitivity of the bases being in the order of thymine, cytosine, adenine, and guanine. A variety of radiolysis products of pyrimidines has been detected but the major product is formed by hydroxyl radical attack on the 5,6 double bond, with both the C5 and C6 being equally susceptible. This action results in the formation of lesions of the 5,6-dihydroxy-5,6-dihydrothymine type. Other products of hydroxyl attack on bases have been detected in the DNA of irradiated cells, e.g. *cis* thymine glycols and 5-hydroxymethyl uracil (61).

### **Repair of radiation injury**

#### *Repair of single-strand breaks*

Single-strand breaks are not chemically homogenous and can have various end groups. Thus, different enzymes systems might be required to carry out rejoining of the different breaks. For example, a break with 5'-PO<sub>4</sub> and 3'-OH end groups could be rejoined

directly by polynucleotide ligase, whereas a damaged sugar would need to be removed and replaced before the gap could be sealed. There are two major pathways for the enzymatic repair of single-strand breaks: a fast, growth medium-independent, pathway that requires DNA polymerase activity and a slow, growth medium-dependent, pathway involving recombination. Fast repair includes all those processes which rejoin DNA single-strand breaks in buffer, while slow repair of single-strand breaks occurs only when bacteria are incubated under conditions that allow growth (61, 62).

#### Repair of double-strand breaks

The mechanism by which bacteria repair double-strand breaks remains unclear. In the relatively radiation-sensitive bacterial species such as *Escherichia coli* very few are tolerated. The maximum number, even in the most resistant strains of *E. coli*, is four. However, no repair of double-strand breaks in wild type *E. coli* has been detected. *Bacillus subtilis* can tolerate a limited amount of double-strand break repair, just two per genome. Some species have a much better repair capacity. As an example, *Deinococcus* species are capable of repairing 150 double-strand breaks per genome (61, 62).

#### **Resistance to irradiation and survival curves**

Resistance to irradiation is expressed as the “*D*-value”, or dose (kGy) required to reduce the microbial population in a given medium (or food) by 10-fold (63). Bacterial populations increase in numbers by doubling; that is, one bacterium reproduces by growing and dividing. When numbers of bacterial cells are converted to  $\log_{10}$ , values are plotted during the active phase of the growth curve (logarithmic growth) and the results form a

straight line. Bacterial populations decline in a similar fashion after being subjected to an environmental stress such as radiation. The kinetics of bacterial death follows a first-order reaction, with the same proportion of percentage of cells killed over time. To allow comparisons between different microorganisms and the same microorganism under different conditions, a decimal reduction value is calculated. This value is the amount of radiation required to reduce the population of a specific bacterium by 90% (1  $\log_{10}$  cycle) under the stated conditions. The calculation is (18):

$$D_{10} = \frac{d}{\log_{10} N_0 - \log_{10} N_1}$$

where  $D_{10}$  = decimal reduction value ( $D$ -value)

$d$  = radiation dose applied

$\log_{10} N_0$  = bacterial population prior to irradiation

$\log_{10} N_1$  = bacterial population after irradiation

The sensitivity of a population of bacteria to the lethal effects of ionizing radiation is usually presented in the form of a survival curve in which the surviving fraction of an irradiated population is plotted on a logarithmic scale against dose on a linear scale. The survival curves of the majority of bacterial species have an extremely small shoulder followed by an exponential loss of viability. Some vegetative bacteria and spore populations, however, have survival curves with a very large shoulder before exponential loss of viability occurs (Fig. 2). In these cells a substantial amount of sub-lethal damage is accumulated before inactivation occurs (61). The  $D$ -value may also be determined by graphing bacterial populations after a series of increasing radiation doses has been applied (e.g., 0.5, 1.0, 2.0, 4.0 kGy). The negative inverse of the slope is equivalent to the  $D$ -value (18):

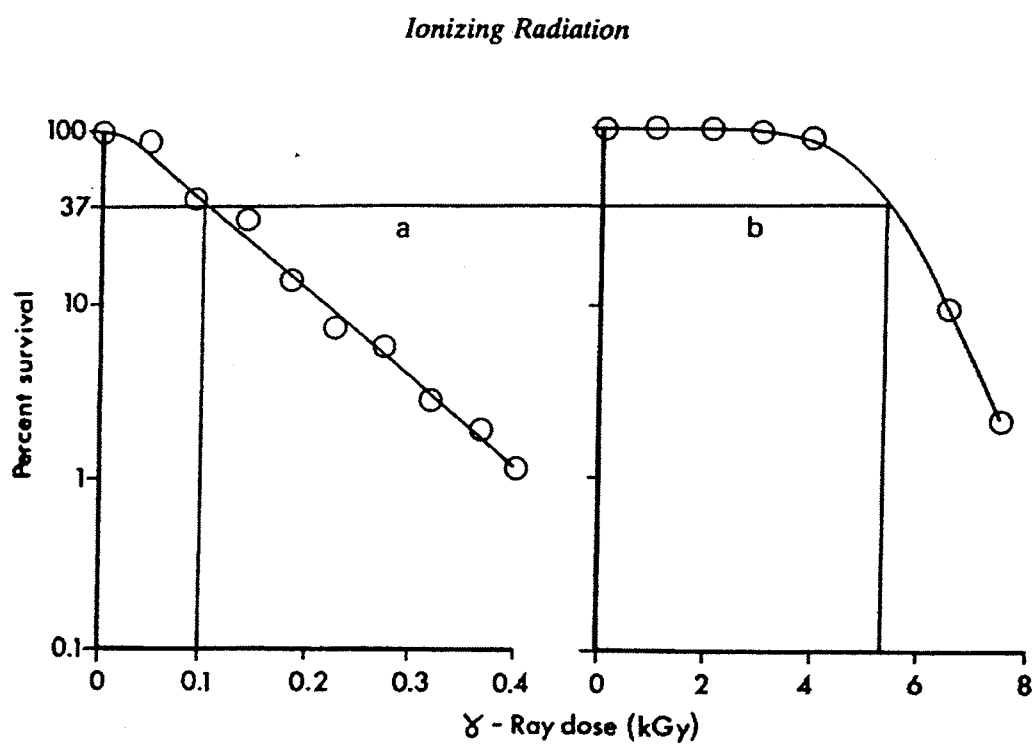


Fig. 2— Gamma-irradiation survival curves of (a) *Escherichia coli* and (b) *Micrococcus radiodurans* (45).

$$D_{10} = -1 \left( \frac{1}{\text{slope}} \right)$$

### **Factors affecting microbial inactivation by irradiation**

Inactivation of microorganisms by ionizing radiation is influenced by several factors such as irradiation dose, numbers and types of microorganisms, food composition and preservation method, temperature, and atmospheric gas composition (53).

#### **Irradiation dose**

Commonly, higher doses of ionizing radiation cause greater destruction of microorganisms. However, microbial destruction at a given dose decreases under anaerobic or dry conditions due to a lower rate of oxidizing reactions that produce free radicals (53).

#### **Numbers, types and age of microorganisms**

Viruses are more resistant than bacterial spores, which are more resistant than vegetative cells. These cells show a higher irradiation resistance than yeast and molds (53). Among bacteria, Gram-positive bacteria are more resistant to irradiation than gram-negatives. Also, in general, spore formers are more resistant than non-spore formers. Among spore formers, *Paenibacillus larvae* seems to possess a higher resistance than most other species. The most sensitive to radiation are the pseudomonads and flavobacters, with other gram-negative bacteria being intermediate (42). The microbial number also affects the irradiation resistance. Large numbers of microorganisms reduce the effectiveness of a given irradiation

dose. With respect to the physiological stage (age) of bacteria, exponential phase cells tend to be more sensitive to irradiation than lag phase cells or stationary phase cells (53).

### **Food composition and preservation method**

Microbial inactivation by irradiation may be affected by the composition of food, including liquid or solids content, protein content and the thickness of the food product (53). Microorganisms in general are more sensitive to radiation when suspended in buffer solutions than in protein-containing media (42). Liquids provide a favorable medium for primary radiolytic products to move and to interact with other primary products or with other components. Dry foods, on the other hand, lacking this medium, are less subject to indirect action and tend to display end results mostly attributable to direct action (95). Diehl et al. (19) reported that proteins exert a neutralizing effect on free radicals, which may explain the relatively high radiation resistance of microorganisms in meats and dairy products. Proteins and other food components, including natural antioxidants such as vitamin C and vitamin E, compete for free radicals formed from activated molecules and the radiolysis of water. This competition for free radicals could minimize the antimicrobial effect of irradiation and compromise the microbial safety and shelf-life of irradiated foods (53).

### **Temperature**

The majority of published research regarding the effect of temperature on the antimicrobial efficacy of ionizing radiation indicates that microbial sensitivity to irradiation is higher at ambient temperatures than at subfreezing temperatures (53). Activation energies of

chemical reactions vary with temperature, and, as a consequence, yields of reactions may be altered (95). Subfreezing temperatures in food cause a reduction in water activity, thus less free radicals are formed, which is associated with increased irradiation resistance of microorganisms in frozen foods. Also, the frozen state of foods inhibits the migration of free radicals to other areas, so they cannot react with more primary or secondary free radicals (53).

### **Atmospheric gas composition**

Atmospheric gaseous composition in contact with microorganisms influences their inactivation by irradiation under specific conditions (53). The radiation resistance of microorganisms is greater in the absence of oxygen than in its presence (42). The type(s) of gas in modified-atmosphere packaging (MAP) may also affect microbial sensitivity to irradiation. Some microorganisms show a higher sensitivity to irradiation when packed in 100% carbon dioxide (CO<sub>2</sub>) than under nitrogen. Some studies have also reported no significant differences in total numbers of certain bacteria that survived irradiation of meat packaged under air versus vacuum (53).

### **Irradiation of poultry meat**

The muscles of healthy, live animals are generally sterile. In live animals, invading microorganisms must overcome a wide array of body defenses before and after entering muscles and organs. Once animal muscles are transformed into meat after slaughter, cutting, and other processing operations, intact or cut muscle surfaces become highly nutritious substrates that are able to sustain rapid growth of contaminating microorganisms. Although



observance of good manufacturing practices (GMPs) may considerably reduce contamination, complete avoidance of contamination is not practical in poultry processing even under optimum conditions. Cross-contamination of poultry carcasses is a common occurrence during scalding, defeathering, evisceration, and subsequent immersion of carcasses in iced water for chilling (56).

The health hazards potential posed by potentially pathogenic microbial contamination and parasites in raw poultry meat are well known and have prompted calls for introduction of modern, preventive system and technologies for their control (56). According to the latest estimate made by the Center for Disease Control and Prevention (CDC), the number of annual foodborne pathogen-linked illnesses in the United States is 14 million, including 323,000 hospitalizations and 5,200 deaths (52). Due to the relatively high numbers of cases of foodborne illness that occur annually, the application of food irradiation continues to gain much attention as a mean to improve the microbial safety of foods. The application of radiation processing to prolong the shelf-life of highly perishable fresh poultry products is also applied to prevent food losses. Irradiation prevents or delays meat spoilage through inactivation or reduction on the numbers of spoilage microorganisms present in the product (56).

The potential economic and health benefits that could be derived from radiation treatment of poultry meat have been reviewed by many investigators (24, 72, 97). The application of irradiation in poultry meat at doses <1 kGy eliminates parasites, and intermediate doses of 1-10 kGy eliminate and/or reduce the number of pathogenic microorganisms (26). A study conducted by Lee et al. (48) involved irradiation of chicken

carcasses at 5-10 kGy, followed by storage at 3-4°C for 41 days. While non-irradiated controls spoiled after only 7 days, irradiated carcasses were microbiologically and sensory acceptable 27 days after irradiation, although acceptability decreased after day 15. Bok and Holzapfel (4) confirmed that *Salmonella* spp. and other pathogens were eliminated in chicken carcasses aerobically packaged in polyethylene bags, and irradiated at 3, 4, 5, or 7 kGy. Carcasses irradiated at 3 kGy and stored at 4°C had a 13-day shelf life.

### **Effects of ionizing radiation on *Listeria monocytogenes***

The high fatality rate (20%) associated with *L. monocytogenes* and its ability to grow at refrigeration temperatures resulted in proposing the use of gamma irradiation in foods to eliminate this microorganism. Several studies have demonstrated the efficacy of irradiation for inactivating this pathogen (53). Patterson (72) irradiated phosphate-buffered saline (PBS) and sterile minced chicken with doses of 0, 0.5, 1, 1.5, 2, and 2.5 kGy; each sample contained four strains of *L. monocytogenes*. The *D*-values in PBS and meat ranged from 0.32 to 0.49 kGy and 0.42 to 0.55 kGy, respectively. The irradiation medium had a significant effect on radiation resistance of the organism, with lower *D*-values being obtained in PBS compared to poultry meat.

Huhtanen et al. (41) inoculated nutrient broth and deboned chicken meat with seven strains of *L. monocytogenes*. Their results were similar to those of Patterson (72) and showed that cultures suspended in broth were more sensitive to radiation than those suspended in meat. Also their study indicated that a dose of 2 kGy was sufficient to destroy 4 log<sub>10</sub> of *Listeria*. In 1989, El-Shenawy et al. (23) determined the radiation sensitivity of three strains

of *L. monocytogenes* in broth and raw ground beef. *L. monocytogenes* strain V7 was the most resistant, and strain Scott A the least resistant in both broth and ground beef. Again, they presented evidence showing that the pathogen was more sensitive in broth (*D*-value of 0.34-0.5 kGy) than in ground beef (*D*-value of 0.51-1.0 kGy).

Similar results were found by Farag et al. (25) when they evaluated the radiation sensitivity of three strains of *L. monocytogenes* in phosphate buffer (PB), trypticase soy broth (TSB), and poultry feed. The strains were more sensitive to irradiation in PB (*D*-value of 0.18 kGy), followed by TSB (*D*-value of 0.21), and more resistant in powdered feed (*D*-value of 0.44 kGy). Also differences in sensitivity among the strains were reported, with *D*-values of 0.21, 0.25, and 0.46 kGy for strains CFPDC, Scott A, and 81-861. These differences indicate that significant interstrain variations in radiation sensitivity exist.

In another study carried on by Varabioff et al. (97) the effect of irradiation and atmosphere-packaging on *L. monocytogenes* in chicken meat was evaluated. Following irradiation at 2.5 kGy no *L. monocytogenes* were recovered in air-packaged conditions during the 15 days of storage at 4°C. In irradiated vacuum-packaged chickens this pathogen was not recovered until day 7 when low levels of the organisms were detected (3.5 log<sub>10</sub>). This observation indicates that not all *Listeria* were destroyed by irradiation at 2.5 kGy, and the surviving cells were able to grow at 4°C in the absence of air.

Thayer and Boyd (94) demonstrated the combined effect of gamma irradiation and MAP for controlling or eliminating *L. monocytogenes* on ground turkey meat. Radiation-sterilized ground turkey meat was inoculated with original inocula which was prepared from the mixture of four strains of *L. monocytogenes*, packaged under mixtures of N<sub>2</sub> and CO<sub>2</sub> and

irradiated at 0-3 kGy. The author reported a statistically significant, but probably not biologically significant, lower (0.39 log) predicted survival of *L. monocytogenes* in 100% CO<sub>2</sub> than in 100% N<sub>2</sub>. Irradiation treatments were significantly more destructive to this pathogen in meat packaged in air than either in MAP or vacuum. A concentration-dependent CO<sub>2</sub> inhibition of *L. monocytogenes* multiplication and/or recovery was observed in meat samples treated at doses >1 kGy.

Patterson et al. (73) conducted another storage study in which raw or cooked minced poultry meat was inoculated with *L. monocytogenes* and stored at 6, 9, 12, and 15°C for up to 28 days in sterile plastic jars following irradiation. In cooked poultry meat stored at 6°C, the lag duration of this microorganism was 1 day in non-irradiated samples and 18 days in samples that had been irradiated at 2.5 kGy. These results imply that even if low numbers of *L. monocytogenes* were to survive the irradiation process, the time required to recover from the irradiation damage would be significant so growth of this pathogen should not be a problem during the shelf-life of the poultry meat.

More recent studies involving the use of electron beam irradiation for improving the microbial safety of foods have demonstrated the efficacy of this type of irradiation for inactivating *L. monocytogenes* (45). Tarté et al. (93) investigated the sensitivity of three strains of *L. monocytogenes* along with *L. innocua* and *L. ivanovii* in ground pork treated with electron beam irradiation. Ground pork was inoculated with one of five strains of *Listeria* and irradiated from 0-1.25 kGy at 0.25 kGy intervals. *D*-values for the three *L. monocytogenes* strains ranged from 0.424-0.447 kGy. *D*-values for *L. innocua* and *L. ivanovii* were 0.638 and 0.372 kGy, respectively. The two pathogenic strains were not

injured significantly at the dose levels used. The authors concluded that the dose range currently considered by the FDA for the irradiation of beef and pork (1.5-4.5 kGy) is adequate for the elimination of *L. monocytogenes* in pork.

### **Physical and chemical effects of ionizing radiation on poultry meat**

Irradiation is the most effective technology for eliminating foodborne pathogens. However, a major concern associated with meat irradiation is lowered meat quality, which is related to off-odor production, lipid oxidation, and color change. Lipids, proteins and vitamins present in the meat are highly-sensitive to irradiation.

#### **Changes in lipids**

Animal fats predominantly contain neutral lipids (triglycerides), phospholipids, sterols and sterol esters, with other lipids in small quantities when detectable. Saturated and mono-unsaturated fatty acids represent the major proportion of neutral lipids in meat. The phospholipids fraction is high in polyunsaturated fatty acids, and it has been established that this fraction, as opposed to the neutral lipid fraction, is primarily responsible for lipid oxidation in muscle foods (33, 57). Under normal physiological conditions, free radicals having one or more unpaired electrons are present in muscle foods and induce lipid oxidation. Lipid oxidation is probably the most widely used measure of oxidative stress in living animals. Ionizing radiation causes the radiolysis of water, which is the largest constituent of meat, and generates free radicals in high concentrations, which attack double bonds in lipid molecules and induce lipid oxidation.

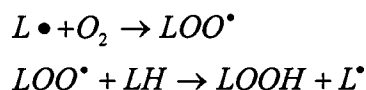
### Chemistry of lipid oxidation

Lipid oxidation is known to proceed by a free radical reaction mechanism involving initiation, propagation/branching, and termination stages.

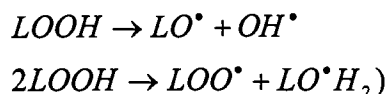
**Initiation:** The first step in lipid oxidation is the removal of a hydrogen from a methylene carbon in a fatty acid (LH) to form a lipid radical (L $\cdot$ ) (57). The formation of lipid radicals becomes easier as the number of double bonds in the fatty acid increases, which is why polyunsaturated fatty acids are particularly susceptible to oxidation. Lipid oxidation can be initiated by OH $\cdot$  or by certain iron-oxygen complexes (e.g. ferryl or perferryl radicals) (57, 60):



**Propagation:** This stage involves the reaction of the lipid radical (L $\cdot$ ) with molecular oxygen to form a lipid peroxy radical (LOO $\cdot$ ). The lipid peroxy radical can remove a hydrogen atom from another unsaturated fatty acid and propagate the chain reaction:

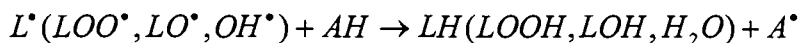
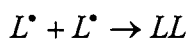
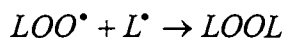
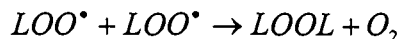


**Branching:** The lipid hydroperoxides (LOOH) formed may undergo homolytic scission to form alkoxy (LO $\cdot$ ) and hydroxyl radicals (OH $\cdot$ ), which are capable of propagating further oxidation and lead to chain branching:



**Termination:** This stage involves the reaction of free radicals to form noninitiating and nonpropagating products. Chain-breaking antioxidants (AH) terminate the free radical chain

reaction by donating hydrogen atoms to free radical species and forming less reactive products:



Lipid oxidation leads to the formation of hydroperoxides, which are very unstable and decompose to form secondary reaction products that adversely affect flavor quality. These secondary reaction products may include hundreds of compounds, such as aldehydes, ketones, alcohols, acids, or hydrocarbons. In many cases, the compounds generated are associated with the term “oxidative rancidity” and are described as having objectionable off-odors and off-flavors (89).

### **Changes in proteins**

Similar to lipids, protein damage due to irradiation is catalyzed by free radicals formed by the radiolysis of water. Damage caused to protein by ionizing radiation include deamination, decarboxylation, reduction of disulfide linkages, oxidation of sulfhydryl groups, breakage of peptide bonds and changes of valency states of the coordinated metal ions in enzymes (17). Major products formed by the interaction of radiation with protein material are carbonyl groups, ammonia, free amino acids, hydrogen peroxide, and organic peroxides (33). Sulfur-containing amino acids along with aromatic amino acids are also susceptible to irradiation damage. The products formed from sulfur-containing amino acids in proteins include methyl or ethyl mercaptan, dimethyl sulfide, carbonyl sulfide and hydrogen sulfide.

When sulfur compounds are submitted to radiation in the absence of oxygen, hydrogen sulfide and other sulfides are formed in large amounts.

Nam et al. (67) reported that meat irradiated in vacuum conditions had the greatest amount of total volatile and volatile sulfur-compounds when compared to aerobically irradiated meat. The amount of volatiles significantly decreased when the meat was exposed to aerobic conditions. On the other hand, in the presence of oxygen the production of ammonia and sulfuric acid also increases (46).

### **Changes in vitamins**

Meat is a great source of water-soluble B complex vitamins (thiamin, riboflavin, niacin, pyridoxine, biotin, cobalamin, choline, folic acid, and pantothenic acid). There is little fat-soluble vitamins in meat (essentially vitamin A). In the case of vitamin radiolysis, the types of possible free radical reactions are determined by the medium in which the vitamins are present. The fat-soluble vitamins would thus be exposed to radicals produced in lipids and the water-soluble vitamins to radicals formed from the radiolysis of water irradiation. For water-soluble vitamins, some may react with hydrated electrons directly or acquire an electron from the other radicals produced in the aqueous medium. The vitamins are most affected by secondary radicals formed by the interactions with the major components which are mostly hydroperoxides (45). Thiamine is the most irradiation labile water-soluble vitamin; however, this vitamin is even more sensitive to heat. Riboflavin, niacin, pyridoxine, biotin, and cobalamin are relative stable to irradiation (33).



### **Changes in poultry meat color**

Ionizing radiation can significantly influence color of meat. It has been reported that turkey breast muscle developed increased red or pink color after irradiation, and the color was stable during refrigeration (69). Millar et al. (55) showed how this irradiation effect on poultry meat was dependent on species, muscle type, and surface measured. During the experiment they performed, the common effect in the muscle of all species tested was increased redness of the freshly cut surface. Nam et al. (66) postulated that the red color results from the formation of CO-myoglobin. The affinity of hemoglobin for carbon monoxide, which is a gas produced during irradiation, is more than 200 times greater than its affinity for oxygen, and could be responsible for the red or pink color in irradiated turkey breast. Nam et al. (66) reported the presence of irradiation generated CO gas in both aerobically and vacuum-packaged meat, but the vacuum packaged meat had a higher concentration. After 2 weeks of storage, the amount of CO decreased in aerobically packaged irradiated turkey breast and a substantial amount remained in vacuum-packaged meat. The CO gas was related to the vivid red color that persisted in the samples when stored up to 2 weeks in refrigeration temperatures. The three forms of myoglobin are deoxymyoglobin, oxymyoglobin, and ferrimyoglobin. All of them are similar, except at the sixth coordination position of heme iron. In deoxymyoglobin it is empty, in oxymyoglobin it is occupied by O<sub>2</sub>, and in ferrimyoglobin it is occupied by water. Oxymyoglobin is more stable toward oxidation than deoxymyoglobin due in part to hydrogen bonding between the bound oxygen and a distal residue of the apoprotein. The oxidation state and the type of ligand bound to the iron atom determine the color and reactivity of myoglobin. The

deoxymyoglobin is the purplish-red pigment, the oxymyoglobin gives the cherry-red pigment, the carboxymyoglobin brings the red-pink vivid color, and ferrimyoglobin, which results from heme iron oxidation, gives a brownish-red and unattractive color (78).

Nam et al. (66) demonstrated that irradiation increased the Hunter *a*-value (redness) of aerobically and vacuum-packaged turkey breast, but vacuum-packaged had stronger intensity. The increased in redness was irradiation dose-dependent and was stable during the 2-weeks storage period. A higher *a*-value indicates a more pink-red color. The lightness (*L*-value) did not change in any of the two atmospheres, and also *b*-values (yellowness) were not affected.

### **Natural antioxidants: Vitamin E**

Antioxidants in food may be defined as any substance that is capable of delaying, retarding or preventing the development in food of rancidity or other organoleptic deterioration due to oxidation. Antioxidants can inhibit or delay oxidation by two ways: (1) either by scavenging free radicals, in which case the compound is described as a *primary antioxidant*, or (2) by a mechanism that does not involve direct scavenging of free radicals, in which case the compound is a *secondary antioxidant* (34).

In general, there is now a greater demand than ever by consumers for foods perceived as natural, fresh-tasting, healthful, and more nutritious (60). Vitamin E is a natural antioxidant classified as a primary antioxidant (34). It is the major lipid-soluble antioxidant, first isolated from wheat-germ and obtained principally from nuts, seed oils and cereals.

Vitamin E is a collective term for eight compounds:  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ - tocopherol, and  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ - tocotrienol (43). They are present, at least in traces, in many food materials.

The most important antioxidant of this group in skeletal muscle is  $\alpha$ -tocopherol, even though  $\gamma$ -tocopherol is the most common in plant foods (16, 101). The prevalence of  $\alpha$ -tocopherol in animal tissues is due to the more than ten-fold preference of tocopherol-binding protein (a liver protein involved in the transport of tocopherols to lipoproteins) for  $\alpha$ -tocopherol than for  $\gamma$ -tocopherol (16). All the tocopherols are derivatives of 2-methyl-6-chromanol onto which a saturated 16-carbon isoprenoid chain is attached at C2 (59). They contain a hydroxyl-bearing aromatic ring structure, which enables them to donate hydrogen to free radicals, and thus act as biological antioxidants (43). Alpha-tocopherol is methylated at C5, C7, and C8 on the chromanol ring, while the other homologous have different degrees of methylation (59).

Tocopherols work as antioxidants by donating the hydrogen of the hydroxyl group to the lipid peroxy radical. The radicals formed from  $\alpha$ -tocopherol are stabilized through delocalisation of the solitary electron over the aromatic ring structure. This radical forms non-radical products, including stable peroxides, which can be reduced to tocoquinones and to tocopherol dimers (Fig. 3). Alpha-tocopherol has also been associated with retarding the decomposition of hydroperoxides (101).

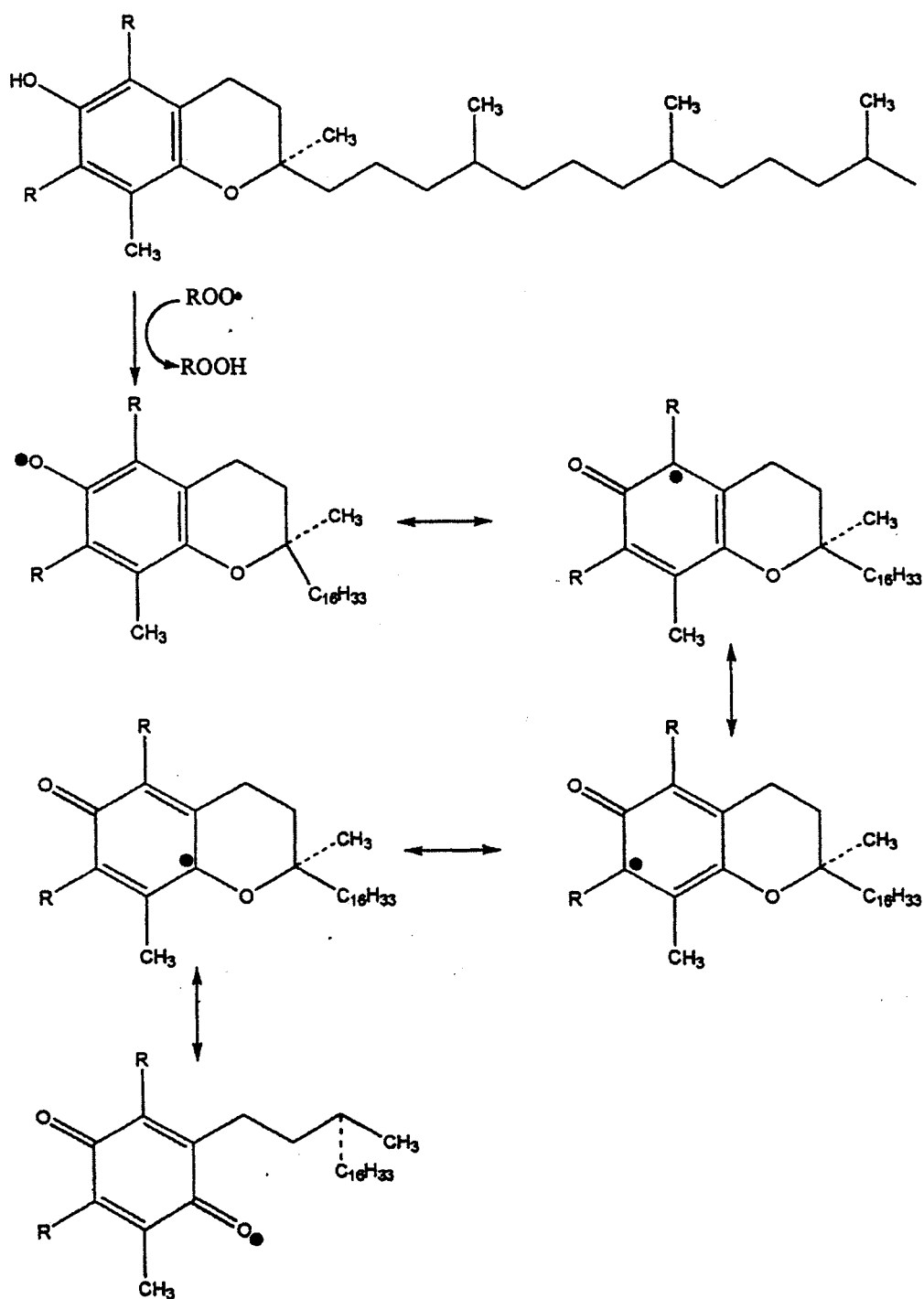


Fig. 3— Resonance stabilization of a free radical by a tocopherol (16).

It has been previously explained how free radicals formed during irradiation affect the quality of the meat, decreasing its quality properties such as color, lipid oxidation, and flavor. Vitamin E inhibits the chain reaction of lipid oxidation, when it reacts with free radicals to form noninitiating and nonpropagating products (57).

### **Incorporation of vitamin E in poultry meat**

Vitamin E is usually added to the animal diets as an ester (e.g.,  $\alpha$ -tocopherol acetate) because of its good stability. Alpha-tocopherol concentration in turkey tissues is considerably lower than those found in broilers and it takes longer for concentrations in muscle to reach saturation. Some researchers have reported that supplementing diets with 600 mg/kg of vitamin E allowed  $\alpha$ -tocopherol concentration to increase at a fast rate up to week 13, after which it reached a plateau. In this case, the highest  $\alpha$ -tocopherol concentration was observed at week 13 in breast and thigh muscle (86). Thus, it takes approximately 13 weeks for  $\alpha$ -tocopherol concentration in breast and thigh turkey muscles to reach a plateau in response to high dietary vitamin E intakes (59). However, other studies have reported that the incorporation of vitamin E occurs after 3-4 weeks of feeding supplemented diets to the animals (68).

Mercier et al. (54) fed turkeys with 30 ppm (controls) and 400 ppm of supplemented vitamin E. Vitamin E content in *M. pectoralis* and *M. sartorius*, of supplemented animals was almost 6 times greater than in the controls. Ahn et al. (1) reported an increase by 3-fold on vitamin E levels in plasma and muscle of turkeys fed 200 IU/kg diet of  $\alpha$ -tocopherol acetate for 105 days when compared with control.

### **Dietary vitamin E effect on lipid oxidation, color, and microbial growth in meat**

Compounds with antioxidant activity such as ethylene diaminetetraacetic acid (EDTA), polyphosphates, ascorbic acid, phenolic antioxidants, and sulfites may inhibit microbial growth, lipid oxidation, and sometimes enhance color stability. It is well known that in the endogenous state, the antioxidant vitamin E delays lipid and oxymyoglobin oxidation and improves meat color and acceptability during display (88). Increased vitamin E concentrations may also have indirect effects on microbial growth through inhibition of by-products generated from irradiation, namely free radicals (88). A few published studies have included measures of generic microorganisms growth in meat from animals fed supplemental levels of vitamin E, and to our knowledge, just one study related the effect of vitamin E and irradiation on microbial growth (90).

#### **Effect of vitamin E in pork meat**

Cannon et al. (7) prepared precooked *longissimus* chops from pigs fed 0 or 100 mg vitamin E/kg and evaluated the samples for lipid oxidation, microbial growth and sensory characteristics. Lipid oxidation was lower in high vitamin E chops than in controls. Off-flavor intensity scores were also more acceptable for high vitamin E samples. For microbial growth evaluation, total plate count (TPC) was determined from samples stored at 2°C for 0, 7, 14, 28, and 56 days. No differences in TPC were found between vitamin E treatments at any given storage time.

### Effect of vitamin E in beef muscle

Chan et al. (12) studied the effect of dietary vitamin E, fed to Holstein steers at doses up to 2000 mg/head/day, on color stability, sensory evaluation and TPC in muscle meat. The total microbial load, irrespective of the muscle specie, was not affected by vitamin E treatment during 12 days of storage at 4°C. The sensory evaluation demonstrated that panelists preferred the appearance of vitamin-E treated meat, and the *a*-value was higher (redder meat) in high vitamin E treatments.

Another study designed to determine populations of aerobic bacteria, coliforms, sorbitol-negative bacteria, and *L. monocytogenes* during display at 4 and 12°C of ground beef patties made with meat from animals fed diets supplemented daily with 1, 1000, or 2000 IU of vitamin E was performed by Cabedo et al. (6). In general, use of high-vitamin E beef versus control beef in patty manufacture had no major difference on any of the microbial populations mentioned above. When the patties were stored at 4°C, *L. monocytogenes* counts fluctuated between 3.7 and 4.5 log CFU/g during the 110 days of display, regardless of level of vitamin E. At 12°C, however, this pathogen reached 6.5 to 7.2 log CFU/g after 4 days in patties made from control and high-vitamin E meat. The overall appearance was also evaluated, and the meat stored at 12°C was considered unacceptable after 2 to 3 days of display. At 4°C the use of high-vitamin E beef resulted in patties with an acceptable color for longer time than patties from control beef. However, all patties were unacceptable after 7 days of display at 4°C.

Eikelenboom et al. (21) examined the effect of dietary vitamin E in color, lipid oxidation and bacterial growth on beef muscle during aerobic and vacuum display for 26 days

at 7°C. Bulls were fed diets supplemented with 2,025 mg of vitamin E. Lipid oxidation during 12 day storage was significantly lower for the supplemented samples. No effect of the vitamin E treatment was observed on  $a^*$ -values. Aerobic plate count increased considerably during the storage period, but this increase did not differ significantly between vitamin E treatments.

Buys et al. (5) studied the effect of dietary vitamin E (up to 500 mg vitamin E per day) in steaks made from beef muscle that were PVC-overwrapped (polyvinyl chloride) and bulk-packaged in MAP with 100% CO<sub>2</sub>, or 20% CO<sub>2</sub>:80% O<sub>2</sub>. Samples were stored up to 42 days at 4°C and display up to 7 days at 4°C. Aerobic plate count, pseudomonad, lactic acid bacteria, *Enterobacteriaceae*, and *Brochothrix thermosphacta* counts were determined. Bacterial counts of rump steaks from either packaged treatment were not significantly influenced during bulk storage or display by supplementation with dietary vitamin E. Both packaging treatments delayed bacterial growth during bulk storage.

### **Effect of vitamin E on ham**

Houben et al. (37) studied the effect of dietary vitamin E and packaging on the color and microbial stability of sliced pasteurized ham. Bulls were fed diets supplemented with 2,025 IU/animal/day. Mesophilic aerobic organisms and lactic acid bacteria had similar growth patterns in hams from the two dietary groups. Same results were obtained from these authors when they analyzed color and microbial stability in minced beef from bulls fed dietary vitamin E supplemented diets (38). Mesophilic aerobic organisms and



*Enterobacteriaceae* had similar growth rates in minced meat from animals in the two vitamin E treatments.

### **Effect of vitamin E in turkey meat**

Wen et al. (100) reported that vitamin E at dietary levels of 300 and 600 mg/kg was clearly protective against iron-ascorbate-induced oxidation. High temperatures during cooking (70°C, 60 min) did not significantly affect the  $\alpha$ -tocopherol content of turkey burgers. In a recent study, Sheldon et al. (86) evaluated the effect of feeding  $\alpha$ -tocopheryl acetate on the oxidative stability, color, flavor, and volatile profiles of refrigerated and frozen turkey breast meat. Lipid oxidation was inversely related to the dietary vitamin E levels. The highest vitamin E treatments (10 and 25x the recommended dose) produced the most typical and acceptable turkey meat flavor with fewer oxidized off-flavor notes for both fresh and frozen samples as opposed to the most oxidized flavor notes detected in the control samples. The high vitamin E samples had significantly lower total aldehyde, hexanal, and pentanal concentrations in comparison to the control samples.

In another study performed by Ahn et al. (1), breast and leg meat patties prepared from turkeys fed diets containing 0, 25, 200, 400, or 600 IU of dl- $\alpha$ -tocopherol, were irradiated at 0 and 2.5 kGy with vacuum and loose packaging. The effects of dietary vitamin E on storage stability were determined. Vitamin E at 200 IU/kg dose decreased lipid oxidation and reduced total volatiles of raw turkey patties after 7 days of storage. This effect was more noticeable when the patties were loosely packaged than when they were vacuum-packaged.

Similar results were found in a more recent study performed by Nam et al. (68).

Turkey breast meat patties, prepared from turkeys fed diets containing 0, 50, 100, or 200 IU of dl- $\alpha$ -tocopheryl acetate per kg of diet, were aerobically packaged and irradiated at 0, 1.5, or 2.5 kGy. Dietary vitamin E at 100 IU/kg diet significantly improved the storage stability of turkey breast, and it was most distinct in irradiated than in non-irradiated meats. Vitamin E significantly influenced the color of aerobically packaged turkey breast meat. Both irradiation and dietary vitamin E increased the redness of the meat, but irradiation had a stronger impact. Thus, dietary vitamin E was effective in stabilizing the color of turkey breast meat during aerobic storage. Irradiated meat produced more sulfur volatiles and aldehydes than nonirradiated meats, and dietary vitamin E reduced these compounds during storage.

Stecchini et al. (90) studied the effect of carnosine added to minced turkey meat on the irradiation resistance of *Aeromonas hydrophila*. Carnosine is an antioxidant naturally found in meat tissue and is different from vitamin E. However, the study described how high levels of carnosine can in fact alter the sensitivity of microorganisms to irradiation. Carnosine was added exogenously to the minced turkey meat and the meat was inoculated with *A. hydrophila* and irradiated. The dose needed to inactivate this microorganism increased from 0.07 to 0.21 kGy in those samples that contained the antioxidant. The authors suggested that carnosine did not have a direct radioprotective effect, but it protected *A. hydrophila* from toxic species produced during meat irradiation. The concentration of carnosine added to the meat (1.5%) was, however, far higher than the physiological content

of vitamin E in meat. Thus, an efficient rate of scavenging resulted due to high concentration of the added antioxidant.

In general, present knowledge indicates that growth rate of generic microorganisms in fresh meat is similar between control and vitamin E-enriched treatments even when modified atmospheric conditions are used. However, the protective effect of antioxidants, based on their chemical composition, and the levels these antioxidants will be added to products that will be exposed to irradiation needs to be examined more in depth with regard to the survival of foodborne pathogens.

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### CHAPTER 3. INFLUENCE OF DIETARY VITAMIN E ON BEHAVIOR OF *Listeria monocytogenes* AND COLOR STABILITY IN GROUND TURKEY MEAT FOLLOWING ELECTRON BEAM IRRADIATION

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#### ABSTRACT

There is growing concern that the free radical scavenging effect of antioxidants added to meats might reduce the antimicrobial effectiveness of ionizing radiation. A study was conducted to determine the effect of vitamin E on the behavior of *Listeria monocytogenes* and color stability in turkey meat following electron beam irradiation. Raw ground turkey breast meat from birds fed diets containing 0 (control), 50, 100, and 200 IU of vitamin E per kg was inoculated with a 5-strain mixture of *L. monocytogenes* to give  $\sim 10^7$  CFU/g. Inoculated samples were irradiated at 0, 0.5, 1, and 2 kGy, and stored aerobically (12 days) or under vacuum (42 days) at 4°C. *L. monocytogenes* survivors were determined by plating diluted samples on Modified Oxford Medium (MOX) and counting bacterial colonies on MOX plates after 48h at 35°C. Meat color was measured using a Hunter Lab colorimeter. Irradiation at 2.0 kGy resulted in  $\sim 3.5$  log reduction of initial numbers of *L. monocytogenes*. There were no significant differences in D-values for *L. monocytogenes* in meat irrespective of vitamin E treatment ( $P > 0.05$ ). Also, vitamin E treatments did not affect growth rate of the pathogen in aerobic or vacuum-packaged samples following irradiation ( $P > 0.05$ ). Compared to controls, irradiated meat from birds fed 100 or 200 IU vitamin E demonstrated significant improvement in color stability (Hunter  $L^*$  and  $a^*$ - values) during aerobic storage ( $P < 0.05$ ).

Dietary vitamin E (100 to 200 IU) has good potential for improving the color stability of turkey meat without compromising the microbial safety of the irradiated product.

## INTRODUCTION

*Listeria monocytogenes* is a psychrotrophic enteric pathogen of major food safety concern. It has been implicated in several outbreaks traced to contaminated cheese, other dairy products, and various types of meat including turkey meat (7). Of all the bacterial foodborne pathogens, *L. monocytogenes* has the second highest case fatality rate (20%) and the highest hospitalization rate (90%) (18). Due to its relatively high fatality rate and the uncertainty of the infectious dose for immune compromised individuals, US regulatory agencies established a zero tolerance for this pathogen in cooked and ready-to-eat foods (28). Therefore, the elimination of this microorganism from foods by use of ionizing irradiation has been proposed. Irradiation of fresh raw poultry meat at doses up to 3 kGy was approved by the FDA to extend shelf-life and to inactivate foodborne pathogens in this meat product (32).

Food irradiation is an effective method for destroying foodborne pathogens. Several studies have demonstrated the efficacy of irradiation for inactivating *L. monocytogenes* in poultry meat (19, 27, 12, 16). Mead et al. (17) demonstrated a 4 log cycle reduction in *L. monocytogenes* on artificially inoculated chilled broiler carcasses following irradiation with 2.5 kGy. Huhtanen et al. (16) investigated the resistance of seven strains of *L. monocytogenes* to gamma irradiation (2 kGy) in deboned chicken meat and reported a 4-log cycle reduction on the numbers of this pathogen. Patterson (27) reported that the radiation

resistance (D-value) of four strains of *L. monocytogenes* in ground poultry meat ranged from 0.42 to 0.55 kGy.

Microbial inactivation by irradiation involves damage to the DNA. Radiation damage to DNA occurs directly via deposition of energy into this macromolecule and/or indirectly by free radicals from the radiolysis of water (19, 22). A major concern associated with free-radical production in irradiated meats is reduced meat quality from off-odor production, undesirable flavors and color changes (9). Since consumer responses to these radiation-induced quality changes are quite negative (1, 11) there is growing interest among meat processors in the use of antioxidants such as vitamin E to alleviate undesirable changes in meat quality during irradiation.

Vitamin E is a natural antioxidant which, when supplied in the diet, becomes incorporated in the subcellular membrane and is effective in scavenging free radicals formed during irradiation (10). Ahn et al. (2) reported a 3-fold increased of vitamin E levels in breast muscle of turkeys when fed 200 IU of vitamin E per kg of dietary treatment. The efficacy of dietary vitamin E in extending the color shelf-life and reducing lipid oxidation in fresh meat has been reported (2, 3, 13). Previous research demonstrated that supplementing the diet of food animals with increased levels of vitamin E was not associated with changes in microbial growth during refrigerated storage of the meat (4, 5). The use of vitamin E seems to be an ideal approach for increasing the quality of irradiated meat; however, the use of this antioxidant in meat poses a major food safety concern because its free radical scavenging action may compromise the lethal action of free radicals against foodborne pathogens during irradiation.

To our knowledge, there are no published reports on the combined effect of ionizing irradiation and dietary vitamin E supplementation on the radiation resistance and growth of *L. monocytogenes* in turkey meat. Accordingly, the main objective of this study was to evaluate the effects of dietary vitamin E on the survival and growth of *L. monocytogenes* in refrigerated (4°C) aerobic or vacuum-packaged ground turkey meat following electron-beam irradiation. A secondary objective was to determine the effect of dietary vitamin E on color stability of irradiated ground turkey meat during aerobic storage at 4°C.

## MATERIALS AND METHODS

### Sample preparation

Twelve-week-old Large White Turkeys were fed diets containing 0 (control), 50, 100, or 200 IU of dl- $\alpha$ -tocopheryl acetate /kg diet. The feeding process lasted until the animals reached 16 weeks of age. At the end of the feeding trial, birds were slaughtered following USDA guidelines (33). Breast muscles were deboned from the carcasses 24 h after slaughter and then were grounded twice using a 3-mm plate. The breasts samples were vacuum-packaged and stored at -20°C for 1 month. After thawing at 3°C for 24 h, samples were prepared by aseptically weighing 10 g of ground meat from each dietary treatment into vacuum bags (Cryovac B-2540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5 – 0.6 g at 100°F, 100% RH/100 sq. in./24 h; oxygen transmission rate = 3 6 CC at 40°F/m<sup>2</sup>/24 h/0% RH).

### **Measurement of vitamin E concentration**

The  $\alpha$ -tocopherol content was measured in breast muscle following the method of Du and Ahn (11). The internal standard used to quantify vitamin E was 5- $\alpha$ -cholestane.

### **Bacterial strains and culture conditions**

A five-strain culture of *L. monocytogenes* including strains H7962 serotype 4b, H7762 serotype 4b, H7969 serotype 4b, H7764 serotype ½ a, and Scott A NADC 2045 serotype 4b was used in this study. *L. monocytogenes* Scott A was obtained from Dr. Irene Wesley at the National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa. All other strains were obtained as clinical isolates from the Bil Mar Foods outbreak of 1998 – 1999 (CDC, Atlanta, GA). Each culture was maintained as frozen (-70°C) stock in brain heart infusion broth (BHI, Difco Laboratories, Detroit, MI) supplemented with 10% glycerol until used. Prior to each experiment, individual stock cultures were transferred twice in 10 ml of tryptic soy broth (Difco laboratories, Detroit, MI) supplemented with 0.6% yeast extract (Difco) (TSBYE) and incubated at 35°C for 20 h.

### **Preparation of inoculum**

Equal amounts of each culture were combined to prepare a five-strain mixture of *L. monocytogenes*. Cells from the mixture were harvested by centrifugation (10,000 x g, 10 min, 4°C) in a Sorvall Super T21 centrifuge (DuPont Instruments, Willmington, DE) and

washed once in sterile 0.85% (w/v) NaCl (saline). The cell pellet was suspended in fresh saline and this suspension was used as the inoculum.

### **Sample preparation and inoculation**

Samples (10-g) of ground turkey meat were each inoculated with 0.1 ml of the 5-strain mixture to give a final cell concentration of  $\sim 10^7$  CFU/g. Each bag was manually massaged for 30 seconds to evenly mix the inoculum into the meat. The bags were then vacuum-sealed using a Multivac A 300/51 vacuum packaging machine, (Multivac Sepp Haggenmuller, GmbH & Co., Wolfertschwenden, Germany) and stored at 4°C overnight prior to irradiation.

### **Irradiation treatment and dosimetry**

Inoculated ground turkey meat samples were irradiated using a Linear Accelerator Facility, which has a MeV CIRCE III Linear Electron Accelerator (MeV Industrie S.A., Jouyen-Josas, France) at Iowa State University. Samples (4°C) were irradiated in duplicate at four target doses (0, 0.5, 1.0, and 2.0 kGy) at a dose rate of 88.1 kGy/min in the electron beam mode at an energy level of 10 MeV. Each target average dose is an arithmetic average of doses determined at the top and bottom surfaces of the irradiated meat samples.

Alanine dosimeters, 5 mm in length by 5 mm in diameter, (Bruker Analytische Messtechnik, Rheinstetten, Germany) were used to determine absorbed radiation doses. The dosimeters were placed on the top and bottom surfaces of one of the duplicate meat samples. Immediately after irradiation, the dosimeters were inserted in a Bruker EMS 104 EPR

Analyzer (Bruker Analytische Messtechnik, Rheinstetten, Germany), which measured the absorbed doses by electron paramagnetic resonance.

### **Microbiological analysis**

After irradiation, approximately half of the packaged of irradiated samples was aseptically opened using a sterile scissors to render the packages aerobic. The open top of each package was loosely folded and closed with a metal clip. Both aerobic and vacuum-packaged samples were held at 4°C and analyzed within 2 h following irradiation to determine numbers of *L. monocytogenes* survivors. Turkey meat samples stored under aerobic conditions were analyzed in duplicate at 0 (2 h), 3, 6, 9, and 12 days of storage (4°C); vacuum-packaged samples were analyzed after 0, 14, 28, and 42 days. Each 10-g sample was mixed with 90 ml of sterile 0.1% peptone water and homogenized for 60 seconds at medium speed using a Seward Stomacher 400 Lab blender (Seward Ltd., London, England). Serial dilutions of the meat slurry were prepared in 0.1% peptone water and 0.1-ml aliquots of appropriate dilutions were surface-plated, in duplicate, on Modified Oxford Medium (MOX) (Difco). All inoculated agar plates were incubated aerobically at 35°C and typical *L. monocytogenes* colonies were counted at 48 h.

### **Calculation of D-values**

The D-value, radiation doses (kGy) that produce 90% reduction in numbers of viable cells, was determined by graphing the  $\log_{10}$  number of *L. monocytogenes* survivors per g versus radiation dose (kGy) using Microsoft Excel 98 Software (Microsoft Inc., Redmond,

WA) (20). The line of best fit for the data was determined using linear regression analysis (26). The D-value was obtained by calculating the negative reciprocal of the slope of the regression curve.

### **Color measurement**

Samples of irradiated ground turkey meat were also evaluated to determine changes in color stability. Color values were measured on the surface of packaged samples using a Hunter LabScan Colorimeter (Hunter Associated Labs. Inc., Reston, VA) that had been calibrated against a black and white reference tiles with the same packaging materials as used for samples. The CIE  $L^*$ -(lightness),  $a^*$ -(redness), and  $b^*$ -(yellowness) values were obtained using an illuminant A (light source). Area view and port size were 0.25 and 0.40 inch, respectively. One color reading was taken from each side of a meat sample.

### **Statistical analysis**

All experiments were conducted twice, and each experiment had two samples per treatment. Thus, means of D-values, bacterial populations ( $\log_{10}$ CFU/g) and Hunter Lab color values were calculated from four replicate samples for each treatment. Analysis of variance (ANOVA) was performed with the General Linear Models procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, NC) (29).

Differences were considered statistically significant at  $P < 0.05$  unless otherwise stated.

Differences among variables were tested for significance using Tukey's honestly significant



different multiple-comparison test. Mean values and standard error of the means (SEM) were reported.

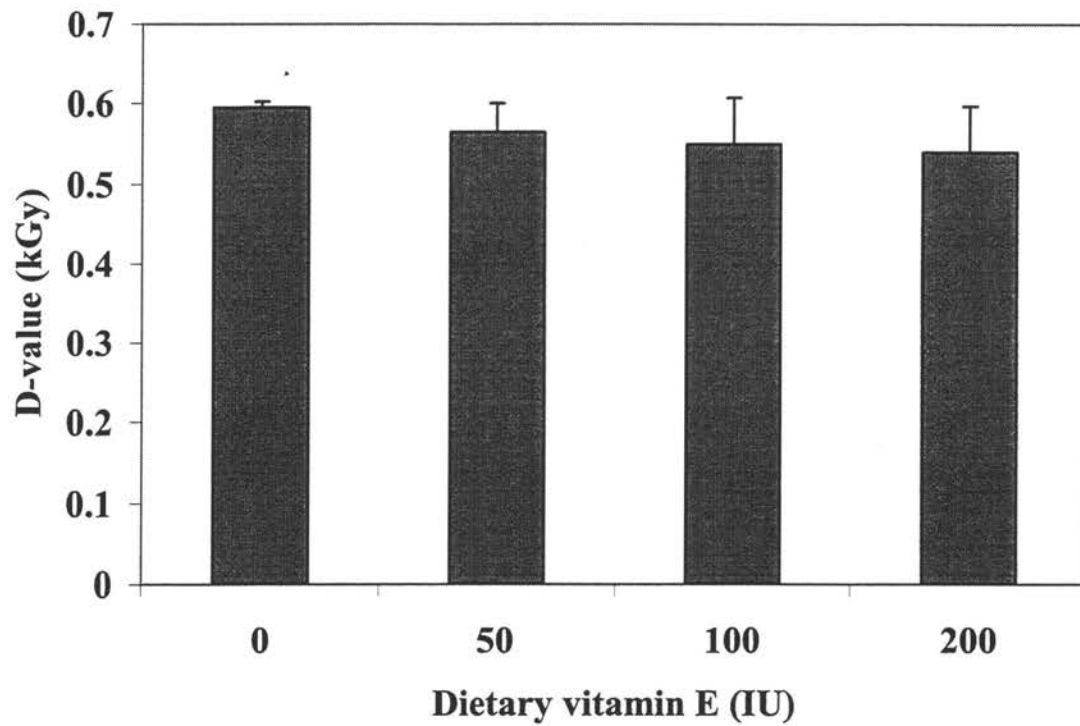
## RESULTS AND DISCUSSION

### Radiation resistance of *L. monocytogenes*

Figure 1 shows the radiation resistance (D-values) of *L. monocytogenes* in ground turkey meat from turkeys fed 0, 50, 100, or 200 IU of vitamin E; D-values ranged from 0.5 to 0.6 kGy. There were no significant differences ( $P>0.05$ ) in D-values of *L. monocytogenes* in turkey meat irrespective of the level of dietary vitamin E used in this study. The  $\alpha$ -tocopherol content incorporated in the breast tissue of birds fed 0, 50, 100, and 200 IU of vitamin E were 0.85, 1.64, 2.24, and 3.47  $\mu\text{g/g}$ , respectively.

Preliminary studies in our laboratory indicated that exogenous addition of 1.5% (w/w) of vitamin E to ground turkey breast meat significantly increased the radiation resistance of *L. monocytogenes* in this meat product. In a similar study Stecchini et al. (31) demonstrated that the addition of the antioxidant carnosine (1.5% w/w) to ground turkey meat resulted in a significant ( $P<0.05$ ) increase in the resistance of *Aeromonas hydrophila* to gamma radiation at 0.5 kGy. These findings indicate that the addition of antioxidants to turkey meat can increase the radiation resistance of foodborne pathogens. Certainly, the concentration of added vitamin E used in our preliminary studies or of carnosine used in the study reported by Stecchini et al. (31) was more than fifty times higher than levels incorporated into turkey breast meat via dietary supplementation when turkeys were fed 200 IU of vitamin E. Therefore, the relatively low level of incorporation or deposition of vitamin E in turkey meat

**Figure 1.** D-values\* for *Listeria monocytogenes* following irradiation of ground turkey breast meat from birds dietary vitamin E



\* Means are not significantly different ( $P > 0.05$ )

from birds fed diets containing up to 200 IU of this antioxidant is insufficient to protect pathogens from the lethal effects of ionizing radiation at 2 kGy.

### **Growth of *L. monocytogenes***

The effect of vitamin E on the growth of *L. monocytogenes* in aerobic- and vacuum-packaged ground turkey meat during storage at 4°C is shown in tables 1 and 2, respectively. When stored in aerobic conditions, the growth of *L. monocytogenes* in non-irradiated meat samples increased from ~7.4 log<sub>10</sub> CFU/g at day 0 to ~8.6 log<sub>10</sub> CFU/g at day 12, irrespective of the vitamin E level (P>0.05) (Table 1). When the meat was irradiated at 2 kGy, a reduction on the initial numbers of *L. monocytogenes* by 3.5 to 3.7 log was observed. Numbers of *L. monocytogenes* survivors decreased by 2.2 to 2.5 log at day 3, irrespective of vitamin E level. After 3 days of storage, the numbers of *L. monocytogenes* increased steadily and reached ~2.0 log<sub>10</sub> CFU/g at day 12. There were no significant differences in growth rate of *L. monocytogenes*, indicating that vitamin E up to 200 IU level had no marked effect on the growth of this pathogen in ground turkey breast meat. Similar results were reported by Cabedo et al. (5), who inoculated ground beef, from animals fed 1000 and 2000 IU of vitamin E, with *L. monocytogenes* and stored the samples aerobically for 10 days at 4°C. A variation about 0.7 log<sub>10</sub> CFU/g in *L. monocytogenes* counts was observed in control and high vitamin E ground meat during the storage period, indicating no major growth or treatment variation. Other studies have also confirmed these results (3, 8, 14, 15).

Table 2 shows a similar trend in *L. monocytogenes* growth in non-irradiated vacuum-packaged meat. The population increased from ~7.4 log<sub>10</sub> CFU/g at day 0 to ~8 log<sub>10</sub> CFU/g

**Table 1.** Effect of dietary vitamin E on populations\* ( $\log_{10}$  CFU/g) of *Listeria monocytogenes* in non-irradiated (0 kGy) and irradiated (2 kGy) ground turkey breast meat stored aerobically at 4°C

Dietary vitamin E (IU)	Storage Time (Days)											
	0 kGy						2 kGy					
	0	3	6	9	12	SEM	0	3	6	9	12	SEM
0	7.42**	7.96	8.24	8.48	8.43	0.20	4.33	1.89	1.00	1.48	2.22	0.41
50	7.29	7.82	8.07	8.09	8.29	0.20	3.83	1.59	1.08	1.08	1.56	0.36
100	7.29	7.81	8.36	8.39	8.56	0.20	3.71	1.37	1.12	1.00	2.03	0.02
200	7.42	8.07	8.48	8.57	8.85	0.20	3.71	1.18	1.00	1.08	1.48	0.09
SEM	0.05	0.10	0.07	0.10	0.10		0.26	0.13	0.02	0.09	0.15	

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\*Means within a column with same irradiation dose are not significantly different ( $P>0.05$ )

\*\*  $\log_{10}$  CFU/g

**Table 2.** Effect of dietary vitamin E on populations\* ( $\log_{10}$  CFU/g) of *Listeria monocytogenes* in non-irradiated (0 kGy) and irradiated (2 kGy) vacuum-packaged ground turkey breast meat stored at 4°C

Dietary vitamin E (IU)	Storage Time (Days)									
	0 kGy					2 kGy				
	0	14	28	42	SEM	0	14	28	42	SEM
0	7.42**	8.57	8.17	7.78	0.2	4.3	3.73	6.24	7.94	0.96
50	7.29	8.33	8.25	7.99	0.2	3.8	3.33	4.46	7.76	1.00
100	7.28	8.6	8.26	8.1	0.3	3.7	3.77	5.32	8.19	1.05
200	7.41	8.63	8.4	8.06	0.3	3.7	3.4	4.95	7.86	1.02
SEM	0.05	0.06	0.04	0.06		0.10	0.09	0.33	0.07	

\*Means within a column with same irradiation dose are not significantly different ( $P>0.05$ )

\*\*  $\log_{10}$  CFU/g

at day 42, irrespective of vitamin E level ( $P>0.05$ ). Immediately after the turkey meat was irradiated at 2 kGy, numbers of *L. monocytogenes* decreased by ~3.6 log, irrespective of vitamin E level ( $P>0.05$ ). At day 14 of storage the population of *L. monocytogenes* did not increase significantly with respect to day 0. The average log was ~3.5, and the dietary vitamin E level did not affect the growth. After 28 and 48 days of storage the numbers of *L. monocytogenes* increased steadily and reached ~8 logs. The increase from day 0 to day 42 was ~4.2 log. Again, growth rate was similar in control and high-vitamin E treatments, showing that high levels of vitamin E did not have an effect on the populations of *L. monocytogenes* in vacuum-packaged turkey meat. These results are comparable to those reported by Buys et al. (4) who vacuum-packaged meat from calves fed diets supplemented with 500 mg of vitamin E per day and then held the meat at 4 °C for 7 days. The researchers found no significant ( $P>0.05$ ) differences in Aerobic Plate count (APC), Pseudomonad count (PC), Lactic acid bacteria count (LABC), *Enterobacteriaceae* count (EC), or *Brochothrix thermosphacta* count (BC) with respect to dietary vitamin E treatment and control (40 mg Vitamin E). The increase in APC, PC, LABC, EC, and BC were similar in both control and high-vitamin E treatment. No differences in microbial load between high-vitamin treatments and controls were found. Cannon et al. (6) also reported similar results. The authors vacuum-packaged precooked pork from hogs fed supplemental vitamin E (100 mg/kg diet) and stored it at 2°C for up to 56 days. Microbial growth was evaluated at every given storage time, and no differences ( $P>0.05$ ) in total plate count between high-vitamin E treatments and control (0 vitamin E) were observed.

## Color Changes

The surface CIE color values of aerobically-packaged ground turkey breast meat were compared based on effects of irradiation dose, storage time, and vitamin E treatment (Table 3). Irradiation increased redness ( $a^*$ -value) of the meat and this increase was irradiation dose-dependent. The redness was also significantly influenced by dietary vitamin E. The color of irradiated breast meat from turkeys fed 200 IU vitamin E/kg diet was visually much redder than the control (0 IU). Nam and Ahn (31) attributed the increased red color in irradiated turkey meat to the formation of a carbon monoxide-myoglobin (CO-Mb) complex. They reported that CO was one of the gas compounds generated by irradiation. Carbon monoxide myoglobin is less readily oxidized to brown metmyoglobin than is oxymyoglobin, because of the strong binding strength of CO to the iron-porphyrin site on the myoglobin molecule. The CO-Mb complex gives a stable bright or light bright red color with consistently high  $a^*$  values (30).

Regardless of irradiation and dietary vitamin E treatments, the color  $a^*$ -values of ground turkey breast meat decreased after 5 days of storage under aerobic conditions. This indicated that the heme pigments were oxidized during the storage period due to the aerobic conditions. The color of irradiated meat, however, was still redder than that of the non-irradiated meat. The lightness ( $L^*$ -value) decreased when ground turkey breast meat was irradiated at 2 kGy. This decrease was significant after 5 days of storage, but the same trend can be observed at day 0 of storage. Low values for lightness implicate darker color in the surface of the meat. The  $L^*$  and  $a^*$ -values are importantly related. Having increased  $a^*$ -values and decreased  $b^*$ -values indicate that the color of the meat was a dark red, and this

**Table 3.** CIE color values of aerobically packaged ground turkey breast meat affected by dietary vitamin E and irradiation during storage at 4°C\*

Irradiation	Dietary vitamin E (IU)									
	Day 0					Day 5				
	0	50	100	200	SEM	0	50	100	200	SEM
<b><i>L* value</i></b>										
0 kGy	54.3	56.1	54.1	54.7	1.2	55.1	55.7	54.8	52.7	1.2
2 kGy	59.0	58.1	53.3	55.6	2.2	57.9 <sub>a</sub>	57.25 <sub>a</sub>	53.11 <sub>bc</sub>	53.2 <sub>c</sub>	1.0
SEM	2.9	0.5	1.4	1.5		1.4	1.6	0.4	0.5	
<b><i>a* value</i></b>										
0 kGy	10.5 <sub>a1</sub>	10.6 <sub>a1</sub>	11 <sub>a1</sub>	11.3 <sub>a1</sub>	0.2	9.52 <sub>a</sub>	9.13 <sub>a1</sub>	9.68 <sub>a1</sub>	11.09 <sub>b</sub>	0.3
2 kGy	13.9 <sub>ab2</sub>	13.5 <sub>a2</sub>	14.6 <sub>bc2</sub>	15.4 <sub>c2</sub>	0.3	9.97 <sub>a</sub>	10.13 <sub>a2</sub>	10.91 <sub>a2</sub>	12.31 <sub>b</sub>	0.3
SEM	0.2	0.2	0.3	0.3		0.2	0.1	0.2	0.5	
<b><i>b*value</i></b>										
0 kGy	18.13 <sub>1</sub>	17.5	17.1	18.0	0.6	16.27 <sub>1</sub>	16.2	17.3	17.1	0.6
2 kGy	16.1 <sub>ab2</sub>	17.9 <sub>ac</sub>	15.5 <sub>b</sub>	18.3 <sub>c</sub>	0.5	19.8	17.56 <sub>2</sub>	17.4	17.5	0.7
SEM	0.3	0.6	0.6	0.6		0.6	1.1	0.5	0.2	

\* Different letters (a-c) within a row are significantly different ( $P<0.05$ )  
Different numbers (1-2) within a column are significantly different ( $P<0.05$ )



color implies that the muscle is healthy and, therefore, very attractive to the consumer. With respect to yellowness,  $b^*$ -values did not change consistently under the effect of irradiation or vitamin E treatments. These results indicate that dietary vitamin E, at 100 - 200 IU/kg diet, would be effective in stabilizing the color of ground turkey breast meat during aerobic display. Similar results have also been previously reported (21, 24, 25).

## CONCLUSIONS

Dietary vitamin E at doses up to 200 IU/kg of the diet of turkeys does not increase the radiation resistance of *L. monocytogenes* in ground turkey breast meat following electron beam irradiation at 2 kGy. However, dietary vitamin E protected the color of the meat (non-irradiated or irradiated), from oxidative changes. The effects of dietary vitamin were dependent upon the vitamin E levels, and at least 100 to 200 IU/kg diet was needed to significantly protect the color in irradiated ground turkey breast meat. Dietary vitamin E (100-200 IU) has good potential for improving color stability of ground turkey meat without compromising the microbial safety of the irradiated product. Future studies should be conducted in order to detect the effect of dietary vitamin E when added in higher concentrations.

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## CHAPTER 4. GENERAL CONCLUSIONS

### CONCLUSIONS

Similar growth rates of *L. monocytogenes* following irradiation in ground turkey breast meat with various levels of vitamin E showed that dietary vitamin E levels up to 200 IU did not protect *L. monocytogenes* from the lethal effects of ionizing radiation in turkey meat. The resistance of the organism to electron beam irradiation, as expressed by *D*-values, was similar in supplemented vitamin E samples and control sample when stored in aerobic conditions. A radiation dose of 2 kGy was enough to reduce the initial population of *L. monocytogenes* by ~3.5 log cycles, irrespective of vitamin E concentration. A significant increase on growth rate was detected in the meat after 14 days of vacuum-storage at 4°C, however, this increase was not affected by the dietary vitamin E levels used in this study. Color was significantly improved by dietary vitamin E in both non-irradiated and irradiated ground turkey meat aerobically stored at 4°C. Increased *a*\*-values in non-irradiated and irradiated samples were detected in the presence of 100 and 200 IU vitamin E. Thus, vitamin E at 100 or 200 IU has the capability of enhancing meat color by increasing the redness of the meat. This is important in extending its fresh appearance and its retail display life. These results offer the meat industry an alternative to reduce color changes in turkey meat during irradiation, thus improving meat quality, without compromising the microbial safety of this nutritious food product.

## RECOMMENDATIONS

1. In the present study dietary vitamin E at doses up to 200 IU/kg diet did not alter the radiation resistance of *L. monocytogenes* in ground turkey meat. Since the level of dietary vitamin E incorporated into the muscle may vary with animal species, similar studies should be pursued to determine the radiation resistance of pathogens in meat from other food animals fed dietary vitamin E.
2. The exogenous addition of natural antioxidants, other than vitamin E, to meat products represents a viable route for the industry to increase the quality of the meat. Some antioxidants may have an antimicrobial effect, thus reducing the microbial population in meat products. It is therefore important to investigate the effect of different natural antioxidants on microbial survival and growth in meat products.
3. The level of antioxidant present on the food system may affect the resistance of microorganisms to irradiation. Some natural antioxidants, such as rosemary extract, may be added to meat products as flavoring agents; therefore, the levels used are much higher than those incorporated via dietary means. More research is necessary to elucidate the effect of high levels of natural antioxidants on survival and growth of microorganisms following irradiation in meat products.

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