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Effects of irradiation on selected pathogens in meat and meat products

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Effects of irradiation on selected pathogens in meat and meat products

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Iowa State University, 1994

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Effects of irradiation on selected pathogens

in meat and meat products

by

An-Hung Fu

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

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DOCTOR OF PHILOSOPHY

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DEDICATION

To my parents, wife, and sons.

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CHAPTER I. GENERAL INTRODUCTION

Food safety is a major concern of consumers and food manufacturers. Beran (1991) stressed that about 25% of foodborne diseases were traced to meat and meat products as sources. Sporadic outbreaks of illness caused from ingesting meat or meat products have evoked wide spread publicity and intensive research on preventing further outbreaks. New technology has modified meat processing procedures, packaging, transportation, and storage methods to provide convenient, various items and also extend shelf-life to gain more profit. Some food pathogens have emerged due to these changes and cause new hazards in these products.

Irradiation has been verified as an alternative to decrease microbial load and to eliminate specific pathogens on meat, without changing nutritive and sensory quality. The USDA/FSIS has approved the use of irradiation to reduce bacterial contamination of raw poultry (USDA, 1992a). More research is required for red meats and their products to demonstrate the advantages of irradiation and gain regulatory approval. Irradiation is a promising means to ensure safety of meat and meat products. The purpose of this study was to evaluate the effects of irradiation at low and medium doses, each at different dose-rates for effectiveness on selected pathogens in meat and meat products. Color, pH, lipid oxidation (TBA), and sensory attributes (color and odor) were also monitored. Products used for this study included: "ready-to-eat" pork chops, cured hams, fresh beef loins, steaks, and ground beef.

Dissertation Organization

A general introduction, pertinent to the whole dissertation is included at the beginning as Chapter I. Chapter II includes a literature review on food irradiation and important meat pathogens. Two individual manuscripts, which are to be published, are individually designated as Chapter III and Chapter IV; followed by general conclusions as Chapter V. A list of literature cited in the general introduction and literature review is at the end of the dissertation.

CHAPTER II. LITERATURE REVIEW

Food Irradiation

History of development

Foods are processed and preserved to increase their variety, palatability, safety, and to extend shelf-life. For many centuries, heating, canning, refrigeration and freezing, dehydration or chemical preservation have been used to fulfill this purpose (Hall, 1989). The form of energy on which the process of food irradiation is based, was discovered just before the start of this century (Dempster, 1985). Irradiation is, therefore, a more recently available means of achieving food preservation.

The early discovery of X-rays by W. K. Röntgen in 1895 and the discovery of radioactive substances by H. Becquerel in 1896 laid the foundation for food irradiation. However, at that time facilities were too expensive to consider practical application (Brynjolfsson, 1989). Most food irradiation studies started in earnest shortly after World War II. The impetus to this research resulted largely from intensive investigations of nuclear energy, which led to the economic production of radioactive isotopes and to evolution of high energy accelerators (Potter, 1978). At that time, use of electron accelerators for preserving food was considered costly and too unreliable for industrial applications.

In the 1950s, the first commercial application of irradiation was by Johnson & Johnson to sterilize medical products using a linear accelerator. However, at the end of the 1950s, Johnson & Johnson replaced the accelerator with cheaper, more reliable cobalt-60 gamma rays (Brynjolfsson, 1989). Research on irradiation of food for shelf-life extension and sterilization was initiated in a few laboratories in the U.S. and Europe

starting in the 1950s and became widespread in some 70 countries in the 1960s (Sigurbjörnsson and Loaharanu, 1989). In the early 1960s, a 10-MeV (million electron volts) linear accelerator located at Risø, Denmark, was operated reliably for industrial sterilization of medical products (Brynjolfsson, 1989). Then, at the Risø facility, several methods for assuring reliable quality control of the irradiation process were also developed. Thereafter, these methods have been modified and used at other research laboratories and at industrial processing facilities.

In the United States, the preservation of food by ionized irradiation (IR) gained much inducement when the late President Eisenhower proposed the "Atoms for Peace" program to the United Nations in December, 1953. Both radiation sources and processing equipment were developed for practical use and stimulated many food irradiation studies. However, early discussion (U.S. Congress, 1958) categorized irradiation applied to food as a food additive, and not a process, and this approach was endorsed by the U.S. Food and Drug Administration (FDA). It meant that the toxicological and other safety concerns would be evaluated the same manner as other food additives. In 1964, the first meeting of a Joint Expert Committee (JEC) of the Joint Food and Agriculture Organization (FAO), the International Atomic Energy Agency (IAEA) and World Health Organization (WHO) recommended that wholesomeness studies be approached from two points of view. First, there should be no pathogenic microorganisms in irradiated food and, second, no significant amounts of toxic products should be formed as a result of irradiation. The effects on nutritional changes and toxicological evidence of safety also had to be considered. This concept continued until the International Project in the field of Food Irradiation (IFIP) was organized in 1970 resulting from an agreement among nineteen participating countries under the joint sponsorship of IAEA, FAO and the Nuclear Energy Agency (NEA) of the Organization

for Economic Cooperation and Development (OECD) (Dempster, 1985). The Joint
FAO/IAEA/WHO Expert Committee meeting held in 1976 concluded that the irradiation
of food was a process akin to other food preservation processes using physical means.
On the basis of a large body of data from toxicological, microbiological and nutritional
studies, five irradiated food items, wheat, potato, papaya, strawberry and chicken, were
recommended for unconditional acceptance. Meanwhile, international interest and
acceptance of irradiated food processing continued to develop (FAO/IAEA/WHO, 1977;
Urbain, 1978). The U.S. army maintained its interest in high-dose irradiation of meat
products such as: shelf-stable bacon, ham, pork, pork sausage, beef, corned beef,
hamburger, catfish cakes and shrimp; the responsibility for low-dose applications
development was transferred to the Atomic Energy Commission (AEC) (Masefield and
Dietz, 1983).

In 1980, national and international interest in irradiation as an alternative to nitrite
in cured meats prompted the transfer of the Army irradiated foods research program to
the Agricultural Research Service of the U.S. Department of Agriculture (Derr, 1993).
Meanwhile, the JEC confirmed that the energy absorbed by irradiated food was much
less than that taken up by heated foods. Consequently, any induced chemical changes
were deemed insignificant. No public health problems arise as a result of food irradiation
and no toxicological testing of food so treated is necessary (Elias, 1980).

The establishment of national regulations on irradiated foods increased during the
1980s in several countries (Loaharanu, 1989). The International Consultative Group on
Food Irradiation (ICGFI) tracks the progress of clearances for irradiated foods in their
Food Irradiation Newsletter (IAEA, 1991). The numbers of countries that approved
food irradiation grew through the 1980's from around 10 countries to over 30 by the end
of the decade.

More and more information on the safety of irradiated food and efficacy of the process has allowed national authorities to sanction this processing method. Currently, 37 countries have approved more than 40 foods or food groups for irradiation. Those were based on the Codex General Standard for Irradiated Foods and the Code of Practice for Operation of Radiation Facilities for Treatment of Foods as published by the Codex Alimentarius Commission in 1983 (Derr, 1993).

Current status of irradiated meat in U.S.

While, the National Aeronautic and Space Administration (NASA) has a petition pending to allow radiation sterilization of beefsteaks for use in the U.S. space program (FDA, 1989) and already has informal permission from FDA to radiation-sterilize beefsteaks for limited use by the astronauts, they would like to formalize the procedure to facilitate future applications (Derr, 1993). The Animal and Plant Health Inspection Service (APHIS) has permitted radiation processing as a quarantine treatment on Hawaiian papayas moving from Hawaii to the U.S. mainland and certain U.S. territories (USDA, 1989a)

U.S. Army irradiation activities were dormant from 1980 to 1991. Interest was rekindled as a result of food distribution problems associated with operations Desert Shield and Desert Storm (Derr, 1993). General William Tuttle, Commander, U.S. Army Material Command, announced the Army's renewed interest in the use of irradiation for two major applications: to extend the shelf-life of refrigerated foods for field use and to produce shelf stable ration entrées. Field feeding is a crucial component of life's quality and irradiation is one technology that can help simplify resupply and reduce dependence on frozen storage, control foodborne pathogens in fresh foods, and at the same time enhance soldier morale. Currently, the Army's program is limited to selection and

economic evaluation of candidate items for field feeding and support for expansion of commercial applications of food irradiation (Tuttle, 1992).

A petition to irradiate poultry with up to 3 kGy (kGy, a standard irradiation unit; one kGy is equal to the absorption of one joule per kg of matter) to eliminate *Salmonella*, *Yersinia*, and *Campylobacter* was submitted in April, 1988. The FDA has approved the use of irradiation to reduce bacterial contamination of raw poultry, including mechanically separated poultry product (FDA, 1990). The FDA is requiring the phrase "Treated with radiation" or "Treated by irradiation" to appear on the labeling of retail packages of irradiated food, along with the irradiation logo. With USDA adding its approval, it has become possible for irradiated poultry to become marketed in the U.S. The USDA/FSIS has approved irradiation of uncooked poultry to control bacteria "that can cause illness when poultry is undercooked or otherwise mishandled" (USDA, 1992a). Since January 1992, Vindicator, Inc., the first commercial food irradiation facility in the U.S., has treated fresh produce, food packaging, and a variety of agricultural products. They successfully marketed irradiated strawberries in the U.S. though there were emotional protests by anti-food irradiation activists initially around the factory (Marcotte, 1992). This plant also started to irradiate fresh poultry at a cost of 2¢/lb. The irradiated poultry has made a debut at four independently owned produce and grocery stores–three in Florida and one in Illinois. A food brokerage firm, Nation's Pride Distributor, Inc., Plant City, FL., distributed the irradiated poultry through a normal distribution system. These products were sold under the "Nation's Pride" label, which was approved through the USDA. By regulation, the label carries the green, international radiation logo. Accompanying the logo was the statement "Treated by Irradiation to control Salmonella and other foodborne bacteria." Also, included was the handling statement "Keep Refrigerated", similar to all fresh poultry (Pazczola, 1993).

Fresh poultry treated within the legal dose range (1.5 to 3.0 kGy) showed elimination of 99.5 to 99.9% of the *Salmonella* organisms and 100% of *Campylobacter* and *Listeria* bacteria (USDA, 1992a; Morrison et al., 1992).

Since each American eats about 70 lb of poultry per year (Katzenstein, 1992), it may be worthwhile to commercialize the irradiation process for poultry. The USDA final rule (USDA, 1992b) indicated poultry products that may be irradiated were fresh or frozen uncooked whole carcasses or parts, including ground, boneless, and skinless poultry, as well as mechanically separated poultry products. Cooked, cured, or poultry products with added ingredients may not be irradiated.

About 91 lb of red meat, including beef, veal, pork, lamb and processed meats are consumed per person per year (National Live Stock and Meat Board, 1991). This is a larger protein source than poultry. To ensure food safety and decrease economic loss, the next step is to seek approval from the USDA to irradiate other red meats.

Irradiation

The term radiation is used for a physical phenomenon in which energy travels through space, even through that space may be empty of matter. Radiation can be considered as either nonionizing or ionizing. Nonionizing radiations possess a level of energy incapable of causing ionization; ionization is the process of ejecting an orbital electron from an atom which leads to a chemical change. The formation of an ion pair of opposite charges results in bond-breakage in a molecule and forms a new substance. If the ionizing radiation carries sufficient energy and can penetrate the nucleus of an atom, a nuclear change can result and may lead to induced radioactivity; this kind of change in food is unacceptable. Therefore, the ionizing radiations used on foods are limited to gamma rays or X-rays of energy up to 5 MeV and to electrons of energy up to 10 MeV

(Urbain and Campbell, 1986). Adequate radiation dose—the quantity of radiation energy absorbed by the food—is the most critical factor in food irradiation. If foods receive less radiation than required, the intended effect may not be achieved and even more hazards occur. On the other hand, an excessive dose may be drastically change food nutritional value and sensory quality rendering the food unacceptable (WHO, 1988a).

Sources Radiation must have the capability of penetrating foods, to be a useful source. Ultraviolet (UV) radiation is essentially absorbed at the surface of materials and therefore it is not practical for food processing. The principal ionizing radiations used in food irradiation include Gamma (γ) ray-emitting isotopic sources, i.e., radioactive ^{60}Co and ^{137}Cs, and machine sources, i.e., a Van de Graaff generator (linear accelerator) and X-ray devices.

X- and γ-rays are the same type of electromagnetic radiation, produced by a beam of high-energy electrons which collide with a dense metal plate. Both have a high penetrating capacity. The difference is that X-ray sources produce the electromagnetic radiation in a broad spectrum which is emitted preferentially in one direction, while γ-rays from isotopic sources are monoenergetic and are emitted in all directions. Both X- and γ-rays have more penetrating capacity than an electron beam, therefore a larger bulk of goods can be irradiated.

The Linear Accelerator Facility (LAF) at Iowa State University is an electron beam; the name itself depicts its irradiation source. The advantage of this type of source is the facility can be turned on and off at will, and there is no radioactive waste disposal problem as when ^{60}Co and ^{137}Cs sources are used. The machine can be adjusted to generate an electron beam or X-rays as needed. The energy level, power level, and conveyor speed can be adjusted to obtain desired irradiation dosage. Electron accelerators are more time-saving than isotopic sources from the standpoint of dose-rate

(Farkas, 1988). Advantages of relatively low cost and a fast process are offset by the fact that accelerated electron beams can penetrate food only to a maximum depth of about 3 inches, which is not deep enough to meet all the goals of food irradiation. An efficient way to get an even dosage in thick samples is to irradiate both sides of the samples.

Irradiation dose units and terminology   The most important factor of the irradiation process is the amount of radiation, or dose, applied. An older unit is the rad, which is equal to the absorption of 100 ergs per gram of matter. The International System of Units is the Gray (Gy), which is equal to the absorption of one joule per kg of matter. One Gy is equal to 100 rads; one Mrad (Megarad, 10^6 rads) is equal to 10 kGy (kiloGray, 10^5 rads). Irradiation processes may be divided into low dose (<1 Mrad, or <10 kGy) and high dose (>1 Mrad, or >10 kGy); or low (<1 kGy), medium (1-10 kGy), and high (10-50 kGy) dose application. At present, the dose of irradiation recommended by the FAO/WHO Codex Alimentarius Commission for use in food irradiation does not exceed 10 kGy. This energy is equal to the amount of heat required to raise the temperature of water by 2.4°C. With this small amount of energy, it is not surprising that food is little altered by the irradiation process, or that food receiving this amount of radiation is considered safe for human consumption (WHO, 1988b).

The dose employed depends upon the objective of the treatment. Three terms are used to describe the different effects of various doses of irradiation (Jay, 1986a).

"Radappertization" is equivalent to radiation sterilization or thermal sterilization (so-called commercial sterility) as in the canning industry. Typical levels are 30-40 kGy. The number of microorganisms will be reduced to very few, if any, that are detectable by any recognized bacteriological testing method.

"Radicidation" is similar to pasteurization of milk, for example. It refers to the

elimination of viable specific non-sporeforming pathogens, other than viruses, to an undetectable level. Typical doses to achieve this effect are 2.5-10 kGy.

"Radurization" may be considered equivalent to pasteurization. The objective of this radiation process is to extend or prolong the product shelf-life and enhance the keeping quality of a food by causing substantial reduction in the numbers of viable specific spoilage microbes. Common dose levels are 0.75-2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains.

The effects of ionizing radiation on food

The radiation energy absorbed by irradiated foods causes a variety of physical and chemical reactions to occur in the treated food. Energy and dose level affect the degree of change directly.

Physical effects Generally, the physical effects of different types of radiation are not important to food irradiation, because maximum dose (10 kGy) has been regulated and proven to be safe. Even if new radioactive materials were to be produced in food by accelerated electrons with high energies, the half-lives of the generated isotopes are so short that radioactivity would not be present in measurable amounts as long as the energy of the electrons was kept below 10 MeV (Hayashi, 1991).

Chemical effects Chemical reactions caused by ionizing radiation are dependent upon dose and the amount of radiolytic products is likely to increase with dose. The chemical changes induced by radiation are influenced by temperature, water content, oxygen concentration, and dose rate. Ionizing radiation penetrates food materials to various degrees depending on the characteristics of the radiation, the level of dose applied and the nature of the food. When ionizing radiation of moderate energy level passes through foods, there are collisions between the ionizing radiations and food

particles at the molecular and atomic levels. Ion pairs are produced when the energy from these collisions is sufficient to dislocate an electron from an atomic orbit. Free radicals result when the collisions provide sufficient energy to break chemical bonds between atoms (Potter, 1978).

The vast majority of the atoms present in food materials are of low atomic number. In a typical "wet" biological tissue, oxygen (~73%), carbon (~12%), hydrogen (~10%), and nitrogen (~4%) account for all but about 1% of the elements present. At the radiation energy levels used, these elements are not convertible into unstable, radioactive isotopes (Coultate, 1989). However, some direct effects can occur when high dose levels are used on food. Changes in the color, flavor, or texture of a food may result from direct collision of a γ-ray or high energy β-particle with specific pigment, lipid or protein molecules. A low radiation dose (<10 kGy) is not sufficient to induce this direct effect. Because the primary goal of food irradiation preservation is to inactivate undesirable microorganisms and enzymes while producing minimum changes in other food constituents, low dose levels (<10 kGy) are usually used to meet this requirement.

In the majority of foods the energy of irradiation is absorbed by the existing water. The incident photon of the γ-ray transfers some of its energy to an orbital electron ejected from the absorbing atom. The residual energy is emitted as a photon of reduced energy. Free radicals, ions, and other products, including $^{\bullet}OH$, $^{\bullet}H$, H_2, H_2O_2, and H_3O^+, formed through radiolysis of water, may cause lethality or sublethality changes in living cells, and are believed responsible for the observed chemical and biological effects of irradiation (Coultate, 1989).

The volatile radiolytic products in beef, pork, ham, and chicken are essentially hydrocarbons and their formation depends on the fat content of the meat, regardless of origin. Electron spin resonance spectra from the four types of meat, irradiated at $-40°C$,

have been found to be essentially identical, indicating the production of common free radical intermediates (Merritt and Taub, 1983). A study of radiation on carbohydrate solutions by Adam (1983) also showed that the radiolytic products and their characteristics were qualitatively identical.

A large number of radiolytic products can be generated; but in fact, the number of chemical reactions following irradiation is smaller than after corresponding thermal processing (Taub et al., 1976). If these radiolytic products are really unique compared with other processing methods, radiolytic compounds could be used as a useful label to identify foods that have been irradiated. Yet, thus far, no reliable analytical method based on this principle has been found for detecting irradiated foods which have been irradiated at the dosages normally used in food processing (WHO, 1988c).

However, attempts to minimize potential indirect effects have been considered. Three approaches have been used with varying degrees of success, depending upon the food material: (1) irradiation in the frozen state, which can decrease free radical production and hinder their diffusion and migration; (2) irradiation in a vacuum or under inert atmosphere can minimize propagation reactions induced by free radicals, due to the exclusion of oxygen from the system; and (3) addition of free radical scavengers to extenuate free radical function (Potter, 1978). Those steps may protect not only foods but also microorganisms and undesirable food enzymes. Thus experimental evaluation of irradiation is necessary for each specific food item.

Dose-rate effects of irradiation

The dose-rates of gamma-rays from commercial ^{60}Co sources can range over 1-100 Gy/min, while those of electron beams from electron accelerators may be 10^3-10^6 Gy/sec. Because of the different dose-rates between these two radiators, very little work

has been done to compare the effects of different types of radiation at the same or different dose-rate in food. Studies are limited to comparisons within same irradiation source at the same dose, which are obtained by different power levels and conveyor speed.

Hypotheses At high dose-rates, theoretically, free radicals can be formed in such high concentrations that recombination of radicals rather than reaction of the radicals with food components is favored, reducing the amount of indirect reactions. An extremely high dose-rate of accelerated electrons will bring about anoxic conditions in the reaction system, because the oxygen in the system is depleted at a rate greater than it can be replaced by diffusion processes of atmospheric oxygen. Oxygen facilitates indirect reactions through the formation of active oxygen groups (free radicals) including superoxide anion radicals and hydrodioxyl radicals, thus the anoxic conditions may reduce the rate of indirect reactions and alter the end result of irradiation. The increased radical-radical reactions and anoxic conditions may be responsible for the difference in the chemical and biological effects observed between gamma-rays and accelerated electrons (Hayashi, 1991; Urbain, 1986a).

Effects on food Food is a complex system and is composed of many compounds such as carbohydrates, proteins, lipids etc. If dose-rate effects are attributed mainly to the increased radical-radical reactions at an extremely high dose-rate, the dose-rate effect involving these primary radicals is not expected to be significant in a food system, because pseudo-first order reactions with the main components will predominate at almost all dose-rate ranges. Reactions of primary radicals with carbohydrates, proteins, and lipids are favored due to high concentrations of these food components and this may produce secondary radicals. Only a small amount of combination of the primary radicals with each other occurs. The majority of the secondary radicals thus formed may

react with the food components again and some of them converted to radicals of greater stability. Thus the radical reaction is likely to proceed in irradiated foods. Consequently, it may be expected that there will be little or no difference in the yields of radiolytic products in foods exposed to different irradiation sources or irradiation at a low and a high dose-rate. On the other hand, if anoxia caused by high dose-rates of radiation plays the only important role in dose-rate effects for chemical reactions, reactions caused by high dose-rates of irradiation will be smaller than low dose-rates and the dose-rate effect in foods will be discernible (Hayashi, 1991).

Effects on microorganisms   Early research (Ley, 1963) suggested that the dose-rate for bactericidal effects of radiation was controversial. And, so far, no conclusion has been obtained from limited studies.

The bactericidal effect of ionizing radiation is caused by damage to biomolecules. However, some damaged DNA and other biomolecules are repaired by the action of enzymes. It is expected that in a low dose-rate treatment, the self-repair actions are of significance in the microorganisms, because damaged biomolecules are repaired before the damage becomes lethal. Beraha (1964) showed that decay of fruits could be better prevented by irradiation at lower doses using dose-rates of 20-40 krad/min than with dose-rates of 1-3 krad/min, and this effect was oxygen-dependent. Dewey (1969) indicated that long term anaerobic conditions prevented the multiplication and repair of cells following irradiation, and did not result in differences at different dose-rates.

On the other hand, extremely high dose-rates result in anoxic atmospheres and decrease the indirect effect by oxygen-related radicals; this will decrease the sensitivity of bacteria to radiation. Also, high concentration of radicals formed and recombined at high dose-rate irradiation, will reduce the radical reactions with biomolecules in microorganisms.

A few reports have suggested that there is no difference in the effect on microorganisms between low dose-rate irradiation and high dose-rate irradiation or between gamma irradiation and electron irradiation. For example, accelerated electrons, X-rays, and gamma-rays had essentially the same bactericidal effect on *Escherichia coli* and spores of *Bacillus thermoacidurans* (Goldblith et al., 1953).

There is no conclusive effect of dose-rate on microorganisms. However, the dose-rate effect can not be clearly observed in a narrow range of dose-rates. It is expected that dose-rate effects can be observed if comparing irradiation at several kGy/hr or lower and irradiation at several kGy/s or higher (Hayashi, 1991)

Wholesomeness of irradiated meat and meat products

Consumers will not accept products which are toxic, lack nutrition, or are unwholesome. Clear evidence of safety of irradiated foods is critical to both regulatory agencies and consumer acceptance. Decades of development and scientific research on the preservation of food via ionizing radiation has indicated that this processing technology has the potential to reduce postharvest losses and chemical applications, thus producing safe foods (Engel et al., 1988).

The term wholesomeness when applied to food irradiation, embodies the concepts of microbiological safety, toxicological safety, and nutritional adequacy (Skala et al., 1987).

Microbiological safety   Microbiological quality has a direct influence on the shelf-life of meat and meat products. Kampelmacher (1983) identified salmonellae, *Campylobacter jejuni*, *Clostridium perfringens*, staphylococcal enterotoxin and certain parasitic infections as the principal pathogens or food disease-related vectors affecting consumers of poultry and meat products world-wide. Studies on control of insect

infestations, transmission of foodborne diseases, effect of spoilage microorganisms, and induced resistance and potential mutations of microorganisms are included in this field (Skala et al., 1987). Most researchers put emphasis on the control of spoilage of meat and meat products, and reduction of pathogenic organisms carried by these foods.

The poultry industry has become increasingly concentrated since the end of World War II. The advantages are improved feed efficiency and decreased production costs; but this also increases the opportunity for the spread of infectious diseases like salmonellosis. Poultry flocks constitute a large reservoir of salmonellae of public health importance and national programs focused on the control of this phenomenon have been suggested (Mulder et al., 1977). The final quality and shelf-life of poultry meat depends on the microbial contamination and storage temperature. One of the possible ways to prolong shelf-life of chilled poultry meat is radurization (Maxcy, 1982; Wierbicki, 1985). The necessary radiation dose is determined by the initial microbial population and by the types of the interactions between radiation survivors (Niemand et al., 1985). Idziak and Incze (1968) indicated that ionizing radiation at 5 kGy reduced salmonellae in fresh eviscerated poultry by 10 log cycles. Katta et al. (1991) studied whole chicken carcasses irradiated by Cobalt-60 using 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy at 3°C, and showed that more than 99% of the microbial load on the carcasses was inactivated by an irradiation dose of 2.0 kGy or more. Ouwerkerk (1980) suggested 3 kGy for a reliable and reproducible effect that would significantly extend shelf-life and reduce pathogen levels.

The potential hazard of trichinosis is one reason some people do not eat pork. Trichinosis is a disease caused by a microscopic parasite, *Trichinella spiralis*, which becomes encysted in the muscle after migrating from the intestine (Engel et al., 1988). The symptoms of trichinosis vary with the level of infection; low levels of infection are

generally asymptomatic. The early stages of infection in humans may be accompanied by diarrhea, nausea, and fever. Once the second generations of larvae migrate to human muscle tissue, additional symptoms may occur, such as muscle stiffness and pain, swelling due to inflammation, fever, and sweating. No cure is available for this disease except for treatment of the distressing symptoms (Murrell, 1985).

The USDA/FSIS has regulations covering the heating, freezing, or drying of pork products which will render the parasite inactive (Jay, 1986b). These are the requirements for pork products that are sold "ready-to-eat." However, irradiation is another potential method to obtain a "trichinae-free" product. Experimental work has indicated the efficacy of gamma radiation at a dose of 0.15-0.30 kGy for the sterilization of *Trichina spiralis* in pork. This result was confirmed by a rat bio-assay which showed that a complete inhibition of the trichina development was enacted by a dose of 0.15 kGy (Kotula et al., 1983). Trichina infestation in fresh pork can be controlled effectively at 0.3 kGy; at higher levels, meat and poultry products could be pasteurized or sterilized and thus safely stored for longer periods.

There is a health concern related to packaging as a result of irradiation of vacuum-packaged meat and poultry products. Vacuum packaging combined with refrigerated storage (<5°C) is the preferred method for extending the shelf-life of many perishable products, including pork. Low dose (<1 kGy) irradiation has the potential of reducing competing microflora and thereby allowing organisms such as *Clostridium botulinum* to flourish in the vacuum-packaged environment. Some strains of *C. botulinum* can produce toxins at temperatures as low as 3.3°C, and concern for the reliability and safety of cold storage of irradiated vacuum-packaged products has been raised. Moreover, spores of *C. botulinum* type E can produce toxins without sensory evidence of spoilage, even though the normal growth of *C. botulinum* causes foul, putrid odors which should

warn consumers. Accordingly, FSIS has prohibited the sale or distribution of any such vacuum packaged products (Engel et al., 1988). In order for this procedure for fresh pork to be acceptable, intensive research is required to prove the microbiological safety of irradiation under 1 kGy applied to vacuum packaged pork.

Fresh beef has a limited shelf-life at refrigeration temperatures. The onset of spoilage is apparent when surface slime, discoloration and the presence of odors become obvious. *Pseudomonas* type microflora are the major causative agent and can be drastically inhibited by irradiation, thus delaying spoilage in meat (Urbain, 1978). However, beef will eventually spoil due to the slow growth of a radiation-resistant group of microorganisms, such as *Brochothrix thermosphacta (Microbacterium thermosphactum)*. Doses of irradiation between 0.25 and 1 kGy can delay microbial spoilage as indicated by a delay in the development of "taint" flavor, by a slower appearance of visible bacterial growth and by a slower rate of increase in the free fatty acid content of the fat (Lea et al., 1960). Irradiation is also effective in reducing numbers of some potential pathogens in vacuum-packaged fresh beef cuts (Niemand et al., 1981) and ground beef (Tiwari and Maxcy, 1972). Some irradiation-resistant microorganisms, especially newly found pathogens, existing in meat samples need to be identified and further investigated.

The shelf-life of meat is controlled by the temperature, level of irradiation, packaging method and chemical preservatives used. Most meat and meat products develop detectable off-odors when radurized at ambient temperatures or above. However, radurization carried out at temperatures of between 0 and 2°C results in less loss of organoleptic properties. Post-radurization storage temperature has an effect on the shelf-life and quality of meat and meat products. Frozen foods generally have better microbial quality than chilled products. However, ice formation in frozen products

provides protection for the bacteria against the effects of irradiation (Matsuyama et al., 1964). The presence of proteins and polysaccharides may also have the same function. Freezing may eliminate the possibility of flavor change or nutrient loss during irradiation, especially when high doses are used.

Radappertization, a commercially sterile process, results in a product which can be stored for years without refrigeration as long as the package is intact. But the palatability of this product can be a major problem due to more radiolytic reactions occurring at a high level of irradiation dose.

Heating processes are often applied together with irradiation because the radiation levels ordinarily used for sterilization do not result in enzyme inactivation which may cause undesirable change in food (Becker et al., 1956). Also, the combination of irradiation and heat treatment is sometimes a useful method to eliminate thermotolerant microorganisms from foods. Thermophilic bacteria, *Bacillus stearothermophilus*, can not be efficiently inactivated by treating them at 100°C. The D_{10} values of *Bacillus stearothermophilus* in aqueous suspension were 1.86 kGy and 2.12 kGy for gamma-rays and electron beams respectively. Both radiation sources enhanced sensitivity of the microorganism to heat treatment depending upon dose (Takizawa, et al., 1990).

Toxicological safety   Toxicological studies have included the vast range of scientific data that the discipline traditionally requires, that is, carcinogenicity, teratology, mutagenicity, anti-metabolite formation, hepatic microsomal enzyme function and general toxicology.

The U.S. Army has a long history of interest in radiation processing of food dating back to 1953, and conducted a number of feeding studies. Raica and Howie (1966) indicated that in no case were any obviously toxic or carcinogenic substances found in the foods tested and concluded that irradiated foods are as wholesome as

nonirradiated foods. Animal feeding study evaluation of radiation-sterilized chicken conducted, initially by the U.S. Army, were continued under the auspices of Agricultural Research Service (USDA/ARS) program (Christopher et al., 1985a; Christopher et al., 1985b). Unfortunately, animal feeding studies have not been entirely definitive because of the extremely low toxicity (or nontoxicity) of the food items. Additionally, animals are unable to consume the exaggerated amounts dictated by experimental design (Urbain, 1978).

Thayer et al. (1987) used irradiation-sterilized chicken, with an average absorption dose of 58 kGy, to do toxicology studies. Results indicated no genetic toxicity or teratogenic effects in mice, hamsters, rats, and rabbits; and provided no definitive evidence of toxicological effects in mammals. Also, no treatment-related adverse effects were observed in dogs, rats, or mice during multigeneration studies, in spite of the fact that the meat was irradiated with a dose almost six times higher than that currently recommended for foods for human consumption.

A 7-15 week human feeding trial with various irradiated foods in China revealed no adverse health effects, including no chromosomal changes (WHO, 1988a).

Nutritional adequacy The side effects of irradiation on the chemical components of food are another concern, similar to other food processing and preparation methods. Nutritional studies have addressed the potential destruction of nutrients, altered bioavailability of nutrients and the evaluation of the impact of such effects on the nutritional contribution of the food item. Radiation studies of isolated nutrients, such as amino acids, vitamins, and sugars, in model systems may be valuable for predicting changes which occur in natural foods, but intricate components in the food matrix and various physical and chemical conditions make the analysis more complicated. However, because of the protective attributes inherent in foods, the sensitivity of

nutritional components is less than that of the same nutrients irradiated in artificial solutions and mixtures.

Chemical change induced via irradiation on carbohydrates, lipids, proteins and amino acids results in modification of food composition and flavor. This has been demonstrated at low- to medium-dose levels in wheat, potatoes, chicken and rice (FAO/IAEA/WHO, 1977; 1981).

The effects of ionizing radiation on carbohydrates are those of hydrolysis and oxidative degradation. From the standpoint of food irradiation, the most important change in polysaccharides caused by irradiation is the breaking of the glycosidic bond. This is caused by free radicals and can result in the formation of smaller carbohydrate units or low-MW compounds such as formaldehyde and glyoxal. Consequently, some foods may change textural characteristics. Another important consequence of radiation degradation of starches is the change in physical characteristics, for example, viscosity (Urbain, 1986b).

Losses of proteins and amino acids by irradiation are small. No significant effect on digestibility, biological value, and net protein utilization, or on amino acid composition has been observed even at a 70 kGy dose (Ley et al., 1969). Frumkin et al. (1973) concluded that use of irradiation on raw beef (6 kGy) and ready-to-eat meat (8 kGy) to retard spoilage would not cause a reduction in its protein nutritional value. However, as with polysaccharides, some depolymerization occurs in aqueous systems. For example, irradiation affects the physical properties (whippability) of egg white, but nutritional value is not largely changed (Urbain, 1986b).

The evaluation of bioavailability of nutrients is more important than the static nutrient content. Research has suggested that antimetabolites to thiamine and pyridoxine might possibly be formed in irradiated pork (Raica and Baker, 1973). These suggestions

resulted in a requirement to test for antivitamin activity using functionality of enzymes which required the vitamins as cofactors. No anti-thiamine or anti-pyridoxine activity has been detected in any radappertized meat tested (Skala et al., 1987)

Effects of radiation on lipids are not unlike those brought about by oxidative and heat processes (Josephson et al., 1978). Lipids, particularly unsaturated fatty acids are much more vulnerable to irradiation than saturated ones. High levels of fatty acid hydroperoxides may be formed when oxygen is abundant. Overall digestibility was unaffected, indicating that lipolysis and absorption of end products were not seriously changed when feeding irradiated (55.8 kGy) lard (Schreiber and Nasset, 1959). Katta et al. (1991) found that most fatty acid levels in breast meat of chicken were not affected by 0.5 to 3.0 kGy irradiation; except for palmitic acid which was decreased and oleic acid which was increased, both significantly, when doses increased. Levels of other fatty acids, notably polyunsaturated fatty acids (linoleic and arachidonic) did not change.

Irradiation dose plays an important role in nutritional changes. At low doses, up to 1 kGy, the loss of nutrients from food is insignificant. Within the medium-dose range (1-10 kGy), some vitamin loss may occur when foods are stored in the atmosphere. At high doses, vitamin loss occurs but can be mitigated by protective measures, such as irradiation under vacuum packaging, processing and storage at low temperatures (WHO, 1988a).

In general, vitamins B_2, D, and niacin are fairly insensitive to irradiation; vitamins A, B_1, E, and K are more easily destroyed. Little work has been done on the effect of irradiation on folic acid, and conflicting results concerning the effects of irradiation on vitamin C in fruits and vegetables have been reported (WHO, 1988a).

Skala et al. (1987) concluded that some vitamin losses were observed in irradiated meats but there was no greater damage than from other processing methods.

The losses do not have a significant impact on a complete balanced diet. Thomas et al. (1981) found that irradiated pork retained more of its thiamine (B_1) than when canned. However, Jenkins et al. (1989) indicated that low-dose gamma irradiation of vacuum-packaged, ground fresh pork caused a dose-dependent, first-order rate of thiamine destruction (R^2=0.99). Storage had little effect on the thiamine content of raw irradiated and nonirradiated pork.

Meat tissue consists primarily of water, protein, fat, and carbohydrate, plus lesser amounts of nonprotein nitrogen-containing compounds and minerals, trace amounts of vitamins and other organic compounds. The complex volatile mixtures produced by these components results in meat flavor while different processing methods exert some influence (Hornstein and Wasserman, 1986). The development of lipid rancidity from irradiated meat products, is one major reason for decreased acceptability (Champagne and Nawar, 1969). Some volatile products degraded by protein precursors may also result in off-flavors (Merritt et al., 1975).

However, Josephson et al. (1978) reported no changes in the content of the normally process-sensitive cystine, methionine and tryptophan in beef; and no significant reduction in the nutritional quality of the protein, lipid, carbohydrate and mineral constituents, regardless of levels of irradiation dose. The overall conclusion was that treatments of up to 5-10 kGy have very little obvious effect on all but fatty foods, except to reduce greatly the rate of microbial spoilage (Coultate, 1989).

WHO (1988a) concluded that chemical analyses and animal feeding studies have shown that the nutritional value of proteins is little affected by irradiation, even at high doses. Animal studies in various species have also demonstrated that irradiation produces only minimal effects to other nutrients.

Legislation and regulation of food irradiation

To ensure the safety of workers and foods, governments need to enact regulations for both irradiation facilities and irradiated foods. Various segments of the United Nations are concerned with the regulatory aspects of food irradiation. These institutions include the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the International Consultative Group on Food Irradiation (ICGFI) of the International Atomic Energy Agency (IAEA), and the Codex Alimentarius Commission (CAC). These groups provide advice and have a strong influence on the actions of member nations regarding regulations for food irradiation.

In the United States the primary federal government agency having jurisdiction over irradiated foods is the Food and Drug Administration (FDA). Other federal agencies also have some jurisdiction on food irradiation, such as U.S. Department of Agriculture (USDA), which regulates meat, poultry, and plant foods; and the Animal and Plant Health Inspection Service (APHIS) which regulates uses of irradiation as a quarantine treatment for fruits to prevent infestation by exotic pests under the Plant Quarantine Act. The Food Safety and Inspection Service (FSIS) regulates the irradiation process in facilities under their inspection using provisions of the Federal Meat Inspection Act and the Poultry Products Inspection Act to ensure the safety and wholesomeness of irradiated meat and poultry products. The Department of Commerce, which regulates marine and freshwater animal foods; the Department of Energy, which regulates irradiation facilities and radionuclide materials; the Department of Labor, which regulates occupational safety and health; Nuclear Regulatory Commission, which controls licensing and regulation of nuclear facilities; National Institute of Standards and Technology, which develops standards for radiation measurements; and Department of Defense, which evaluates

applications for field feeding (Urbain, 1986c; Derr, 1993) are other agencies with some jurisdiction.

Food irradiation plants should be licensed by the government agency responsible for the regulation of irradiation applications and installations. Such a license should be granted only after a thorough investigation has established the plant is safe and appropriate, the design and construction meet applicable standards, its operators are fully trained, and the operating plans and procedures give all necessary attention to the requirements of radiation safety. Regular performance checks should examine the quality of the products being irradiated to ensure proper dose of radiation is being delivered for the intended effect (WHO, 1988d).

The Codex General Standard for Irradiated Foods and the Recommended International Code of Practice for the Operation of Irradiation Facilities Used for the Treatment of Food, both from the Codex Alimentarius, Vol. XV, 1984, provide authoritative guidelines that are recognized by regulatory authorities and industry around the world as a basis for safe and effective radiation practices (WHO, 1988d).

Advantages

Research on irradiation has been extensively conducted with both private and government programs in the U.S. Today, there is a world-wide activity at various levels involving food irradiation and many experts feel it is a process ready for use.

Research done since the mid-1940 has demonstrated that treating foods with irradiation can provide numerous advantages (Urbain, 1989):

(1) Preserves foods: Food irradiation is particularly effective in controlling foodborne spoilage microorganisms; it has the potential to reduce the incidence of foodborne diseases. All organisms present in the food can be inactivated to secure long-

term preservation or a fraction of them can be inactivated to secure limited extension of product life; for example: reduction of foodborne *Salmonella*, which would reduce the frequency of salmonellosis; irradiation of pork to control trichinosis and toxoplasmosis; reduction of parasites such as tapeworm in beef.

In addition, irradiation can extend the market life of produce, which after harvest continue to be physiologically active, by delaying their maturation, senescence, or sprouting, especially for fresh fruits and vegetables.

(2) Decontaminates foods: The decontamination can improve the hygienic quality of the foods and prevent the potential of health hazards. Meats and seafood can be decontamintated of bacteria and parasites. Bacteria and insects can be eliminated from dried food materials, such as spices and vegetable seasonings.

(3) Improves quality: Chemical alterations can be achieved through action of the radiation on the food or through modification of an associated biological process. The amount of radiation absorbed by the food depends on the dose applied, and this is the most important process parameter for securing the desired effect.

(4) Reduces chemical and toxic residues: Reduction of nitrite used in processed meat products for protection against botulism is one example of reduced use of an additive which has generated much concern. Other chemical preservatives may also be decreased, thus resulting in lower chemical residue in food.

The lethal action of ionizing radiation on living organisms has been attributed to alteration of the DNA molecule but very few other changes occur. The limited chemical change in a food caused by irradiation is well correlated with the limited amount of energy utilized in irradiation. Radiation chemistry research, plus animal testing, has established that irradiated foods are toxicologically safe for human consumption. The

microbiological safety of irradiated foods was assured through application of results of investigations in that field.

(5) Maintains full nutritive value of foods: Studies have shown that irradiation caused no change in the macronutrients and only insignificant ones in the micronutrients (vitamins). In short, irradiated foods are nutritious.

(6) Maintains sensory quality: Because of the very small amount of chemical change occurring in irradiated foods, generally no detectable sensory change results (depending on dose). Whereas heat sterilization severely degrades the texture of meats, for example, radiation sterilization produces very little texture change.

Consumer acceptance

The uncertain acceptance of consumers and food processors impede the further development of this food preservation technology. Although some 34 countries have granted approval for radiation processing of about 30 food items, industry has been slow to expand the use of radiation. Hall (1989) concluded that the reasons for slow progress of commercialization of food irradiation were: regulatory and safety aspects of the process, equipment cost factors, and obtaining the necessary consumer acceptance. Negative public attitudes towards virtually everything associated with radiation are found all over the world. Apparently, there is wide public misunderstanding of what the process is, how it works, and what it will and will not do. A major misconception is that food processed by radiation becomes radioactive. Also, Leemhorst (1990) indicated that irradiation is often mistakenly believed to be used: (1) to mask bad quality, (2) to improve spoiled foods, (3) to suppress spoilage indicators like odor and taste, and (4) to relax Good Manufacturing Practices (GMP). In fact, irradiation only can delay microbiological spoilage, it can not reverse spoilage processes and will not remove or

modify any spoilage indicators from the food. And, most importantly, GMP are still needed to control overall quality of irradiated food.

Irradiation is often presented to consumers as an alternative to chemical treatment. An early survey compared consumer preference for chemical preservatives and irradiation (Harris, 1985). In the total sample there was no difference in preference, whereas for those who had heard of irradiation, 39% preferred that technology compared to 24% who preferred chemical preservatives. This suggested that information about irradiation would improve acceptance, because consumers had more information to make a judgment, thus enhancing the importance of communication and education.

Schutz et al. (1989) studied consumer attitude toward irradiated foods. If consumers were informed that irradiation was used to resolve problems for poultry (*salmonella*) and pork (trichina), a majority of the respondents (58.5%, 56.7%) indicated they would choose irradiated meats. Approximately 18-19% indicated they would choose the non-irradiated products.

Once consumers understand that irradiation of food is not harmful, does not make food radioactive, and that foods processed by irradiation may be safer and more nutritious than those prepared by conventional means, the question of whether irradiated food should be labeled in some special way becomes a nonissue. Indeed, consumers may gain a positive image of this technology if the proper education programs are utilized (Korwek, 1983). Thus, labeling a food as having been irradiated might eventually have promotional value.

In recent years, the public has become much more aware of the threat of foodborne illness caused by pathogens. Food safety is one of the most important requirements by consumers. Radiation processing may be a promising solution to conquer food safety problems which exist all over the world.

Important Meat Pathogens

Foodborne illness

The United States produces some of the safest and the most nutritious food in the world. However, there is still an enormous number of outbreaks of foodborne illness and many people die of them annually. Foodborne illness is an illness or disease that occurs as a result of eating food that serves as a carrier of the causative agent. Statistics on the occurrence of foodborne outbreaks are compiled by the Centers for Disease Control (CDC) in Atlanta, Georgia. A foodborne disease outbreak is defined by the CDC as an incident in which two or more persons experience a similar illness after ingesting a common food, and epidemiological analysis implicates the food as the source of the illness. A few exceptions to that definition exist; for example, one case of botulism or chemical poisoning constitutes an outbreak. Outbreaks of known etiology are those for which laboratory evidence of a specific agent is obtained and specified criteria are met. Other outbreaks of unknown etiology are subdivided into four subgroups by incubation period of the illnesses: <1 hour (probable chemical poisoning), 1-7 hours (probable *Staphylococcus* food poisoning), 8-14 hours (probable *Clostridium perfringens* food poisoning), and >14 hours (other infectious or toxic agents) (Bean et al., 1990).

There are three types of microbial foodborne disease (Zottola and Smith, 1990):

(1) Food infection: An infection occurs when live pathogenic bacteria are ingested together with food. The bacterial cells can colonize and grow in the intestinal tract of the host, then cause epithelial tissue damage with resultant symptoms. Only few cells can induce foodborne infection. Examples of food infections are salmonellosis,

shigellosis, listeriosis, yersiniosis, campylobacteriosis, and *Vibrio parahaemolyticus* gastroenteritis.

(2) Food intoxication: Intoxication is caused by ingesting a toxin, which is produced by the contaminating microorganism on food. Whether viable microorganisms exist or not, the host will become ill while the toxin is active. Examples of food intoxications are botulism, staphylococcal food poisoning, and *Bacillus cereus* gastroenteritis.

(3) Food intoxification: Intoxification is a combination of the above two categories. High number of pathogens in the food is required. Cells are ingested by the host with the food and continually grow in the gastrointestinal tract with toxin production, thus inducing symptoms of the illness. Examples of microorganisms that cause food intoxcifications are *Clostridium perfringens*, enterotoxigenic *Escherichia coli*, and *Vibrio cholerae*.

Intrinsic and extrinsic factors which influence the growth of microorganisms in food also affect pathogenicity of microorganisms Various pathogens possess different ability to tolerate competition in the food environment. Toxin production usually requires more specific conditions than does growth of microbial cells. Thus only predominant flora can cause illness in most cases.

Etiology

Foodborne illnesses result from many different agents, such as pathogenic bacteria and/or their toxins, viruses, fungi, parasites, chemicals, and natural toxins present in plants and animals. From the CDC Surveillance Summaries (Bean et al., 1990), confirmed foodborne disease outbreaks/cases caused by bacterial pathogens (66%/92%) were much higher than those caused by chemical agents (26%/2%).

However, the public and experts do not agree on importance of the sources of food hazards. Lee (1989) indicated that the public have much more concern about new food chemicals and pesticide residues, but experts concur that microbial contamination poses the greatest risk. Various factors, including inappropriate reports by the mass media, enhance the different perceptions by the public and the experts.

The three most prominent bacterial agents are *Salmonella*, *S. aureus*, and *C. perfringens* in the U.S., Canada, and Europe in general (Banwart, 1989). More recent data (Bean et al., 1990) showed that *Salmonella* was still responsible for the greatest numbers of outbreaks (57%) from 1983 to 1987, followed by *C. botulinum* (12.3%), *S. aureus* (7.8%) and *Shigella* (7.3%). *Listeria monocytogenes* was not reported as foodborne pathogen during this period. In 1992, *Salmonella* and *Campylobacter* spp. together accounted for about 4 million cases of illness in the U.S.; followed by *Listeria monocytogenes*, which caused 1500 cases of illness in the U.S. (USDA, 1993).

The most common types of chemical food poisonings were ciguatoxin and scombrotoxin, which are found in fish. Poisonous chemicals, heavy metals, pesticides and detergents may inadvertently enter the food chain and cause problems. Excessive amounts of food additives (e.g., MSG) can also cause illness, especially in sensitive individuals.

Trichinosis, caused by the nematode, *Trichinella spiralis*, was the most common foodborne parasitic infection. The primary reason is attributed to undercooked pork.

Hepatitis A was the primary viral infection transmitted by food and/or water, while Norwalk and other viruses may cause other viral infections. The limitations of current laboratory techniques result in difficulties for isolating and identifying viruses (Zottola and Smith, 1990).

Economic impact

The CDC estimated that approximately 6.5 million cases of foodborne diseases of microbiological and parasitic origin occur in the U.S. each year. The major symptom of these diseases is diarrhea or dysentery. In the U.S., about 68.7 to 275 million cases of diarrheal disease episodes from all causes occur annually. Furthermore, the economic impact caused by food supplier losses, law suits, and consumers' medical and hospital costs is also tremendous (Todd, 1984). Approximately 8.4 billion dollars were estimated as the cost of foodborne illnesses (Todd, 1989).

Pathogens of meat

About 25% of foodborne diseases have been traced to meat and meat products as sources (Beran, 1991). Bryan (1988) indicated, from 1977 to 1984 in U.S., meat (23.3%) and poultry (9.8%) were the second and third most common sources of foodborne illness, while seafoods (24.8%) caused most of the outbreaks. More recent statistical data (Bean et al., 1990) , from 1983 to 1987, showed that shellfish and other fish (22.4%) were the leading foods implicated as sources of transmission. Outbreaks from both meat (13.1%) and poultry (5.2%) were decreased. But the category of foods listed as "other" (unconfirmed sources) which was less than 5.0% for the earlier period (1977-1984), was 30.8% for the more recent period (1983-1987). Zottola and Smith (1990) suggested that some new emerging pathogens may evade current laboratory identification and contribute substantially to the "unconfirmed sources" category.

In recent years, other microbes that are capable of causing foodborne illness have been observed. They are termed "emerging" because of the improved isolation and identification methods have been developed by scientists. In addition, advanced food technologies including new processing methods, large-scale and high-speed food

production, different preservation measures, and new packaging methods result in changes of spoilage microorganisms which may permit emerging pathogens to grow and contribute to foodborne illness outbreaks (Zottola and Smith, 1990; Scott, 1988). Cox (1989) identified seven reasons why pathogens emerge on foods. These were: (1) changes in eating habits; (2) changes in perception and awareness of what constitutes hazards, risks, and hygiene; (3) demographic changes; (4) changes in primary food production; (5) changes in food processing technology; (6) changes in handling and preparation practices; and (7) changes in the behavior of microorganisms. Hooper (1989) emphasized that changing processing and packaging conditions may permit traditional and less common foodborne pathogens (i.e., *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica*) to survive and endanger food safety. For precooked meat products and convenience foods, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Listeria monocytogenes* were the so-called emerging pathogens of particular concern due to ideal nutrient composition and lack of competitive or repressive organisms (Raccach and Baker, 1978; Webb et al., 1990). Such developments emphasize the need for food processors and food service establishments to continue vigilance in microbiological food safety. In one of the most important areas, the consumer needs to understand how to handle, cook and store foods appropriately.

General effect of irradiation on microorganisms

The D_{10} value is an indicator of the degree of radiosensitivity of a given bacterium under specific conditions. The D_{10} value is calculated by the following equation:

$$D_{10} \text{ (kGy)} = \frac{U}{\log_{10}A - \log_{10}B}$$

where: U is treatment dose in kGy; $\log_{10}A$ is initial bacterial population; and $\log_{10}B$ is surviving bacterial population. From this formula the efficiency of a radiation treatment on a bacterial population can be calculated.

Microorganisms under evaluation could be inoculated in solution or food, but some difference may occur due to different media used. For example, if ground beef is used as a medium, the D_{10} value is likely to be higher than if a solution is used. It is thought that less water exists and that proteins reduce the level of oxygen thus protecting microorganisms against radiolytic reactions which are largely responsible for lethality.

A study conducted by Maxcy (1983) indicated that microbial flora after irradiation would shift from Gram-negative to Gram-positive. Lefebvre et al. (1992) confirmed that dominant groups of bacteria shifted from Gram-negative bacilli to Gram-positive cocci as the irradiation dose increased. The majority of the microorganisms studied (22/27) had D_{10} values less than 0.5 kGy. Thus low to medium doses of irradiation are enough to decrease microbial loads and extend shelf-life. These authors treated ground beef (initial total counts were 7 log CFU/g) with gamma radiation at doses of 1.0, 2.5, and 5.0 kGy which resulted in extended shelf-life at 4°C of 4, 10 and 15 days, respectively.

In relation to the "hurdle concept" (Leistner and Rödel, 1976) and to the interest in "minimally processed and extended shelf-life chilled foods" (Livingston, 1985), the combination of different inhibitory factors can result in considerable improvement in the microbial quality of food. The flora shift and shelf-life extension of irradiation, for example, were enhanced by vacuum (Ehioba et al., 1988) or modified atmosphere packaging.

Effect of irradiation and acidulants   Decreasing pH by organic acid rinsing or dipping of meat, or by adding food-grade acidulants has been found to be effective in inhibiting certain microorganisms without affecting organoleptic

characteristics (Hamby et al., 1987; Dixon et al., 1987). The combination of irradiation and acidulants may provide a significant hurdle to growth of microorganisms. Niemand et al. (1983) reported that a combined treatment of lactic acid (pH 5) and irradiation (2.5 kGy) extended shelf-life of minced beef longer than irradiation alone. Microbiological effects on an irradiated vacuum-packaged, minced meat product prepared from pork and beef with spices and cereal fillings were monitored by Farkas and Andrássy (1993). They used a gamma irradiation dose of 2 kGy, with and without reduction of pH to 5.2 or 5.3 by adding glucono-delta-lactone (GDL) or ascorbic acid, individually. The irradiation alone caused 2 log reduction of aerobic plate count and 4 log reduction in the Enterobacteriaceae count. The result agreed with Ingram and Farkas (1977), and Tarkowski et al. (1984a) on the radiation sensitivity of Enterobacteriaceae. The inhibition effect on aerobic plate counts, by adding acidulants, was enhanced throughout the storage period at 0-2°C for 4 weeks. Lactic acid bacteria appeared to be more radiation resistant and became the dominant flora during storage. This may be considered as a built-in safety factor against the growth of certain pathogens (Gombas, 1989). Combination of pH-reduction and irradiation prevented growth of Enterobacteriaceae even at 10°C incubation.

Effect of irradiation and modified atmosphere packaging Modified atmosphere packaging (MAP) including vacuum and gas packaging, has been used extensively by the meat industry to extend the shelf-life of fresh meat (Young, 1988). By using MAP and refrigerated storage, pork or beef can attain 4-8 weeks of shelf-life, depending on the initial microbiological quality and on the gas mixture used. But there are concerns that these atmospheres may permit toxin production by *Clostridium botulinum* in some modified atmospheres, particularly under conditions of temperature abuse (Silliker and Wolfe, 1980; Hauschild et al., 1985). It might be expected that shelf-

life would be extended when MAP and irradiation processing are combined. However, the risk may be high if low-dose irradiation is applied, because of elimination of competing microorganisms. The results of studies to date on the effect of O_2 and CO_2 in modified atmospheres on toxin production by *C. botulinum* are inconclusive. Studies of combination effects of MAP and irradiation have been limited. Lambert et al. (1992) indicated that 1 kGy reduced both psychrotrophic and mesophilic bacterial counts by two log cycles and inactivated most of the Enterobacteriaceae while lactic acid bacteria were largely unaffected. Although the 20% O_2/80% N_2 in the package headspace extended the shelf-life (31 days) compared with 0% O_2/100% N_2 (21 days), 20% O_2 adversely affected the organoleptic qualities of irradiated pork. Additionally, toxin production by *C. botulinum* occurred faster in samples initially packaged with 20% O_2, compared with samples packaged with 0% O_2 (Lambert et al., 1991). They found 1 kGy irradiation was significant in delaying the time until toxin detection in samples initially packaged with 20% O_2 but not in other treatments. However, on a positive note, all samples were spoiled before they became toxic.

Low levels of total counts and of pathogenic organisms are indicative of a good quality product. Certain doses of irradiation may kill part of the microorganisms but may only injure some microorganisms, which could recover during storage if parameters permit. Combined treatments involving heating, chemical additives, or modified atmosphere packaging, and appropriate refrigeration have improved shelf-life. However, cold-tolerant microorganisms, especially some pathogens, still can survive at refrigeration temperatures, and may cause food illness. More research is required to ensure the safety of these products especially when the predominant spoilage organisms are inhibited.

Listeria monocytogenes

Characteristics   *L. monocytogenes* is a Gram-positive, motile, non-sporeforming rod, 0.4-0.5 µm in diameter and 0.5-2 µm in length, with rounded ends. It is flagellated and motile in a characteristic tumbling or slightly rotating fashion. Although optimal temperatures for growth are 30-37°C, motility is greater at 20-25°C. This microorganism is a psychrotroph and a facultative anaerobe, and is able to survive at a wide range of temperatures from 4°C to 45°C, as well as in aerobic and modified atmospheres. Also it can grow at a wide range of pH from 5.0 to 9.0.

All *Listeria* spp. give a positive catalase reaction and a negative oxidase reaction, and produce acid but no gas from dextrose, esculin, or maltose. *L. monocytogenes* produces acid but no gas from rhamnose as do some strains of *L. innocua*, *L. welshimeri*, and *L. murrayi*. *L. monocytogenes* can not utilize xylose and mannitol. The rhamnose and xylose reactions are important in differentiating *Listeria* species.

The CAMP test is used to confirm or determine hemolysins and is therefore useful in differentiating *Listeria* species. *Staphylococcus aureus* (a ß-hemolytic strain) and *Rhodococcus equi* are streaked vertically onto a blood agar plate, and the test cultures are streaked horizontally between them. *L. monocytogenes* and *L. seeligeri* enhance hemolysis in the vicinity of the *S. aureus* streak, while *L. ivanovii* enhances hemolysis in the vicinity of the *R. equi* streak (Lovett, 1988).

Source   *L. monocytogenes* is commonly found in the environment including water, soil, feces, feed, and forage. It has been isolated from domestic farm animals, humans (Silliker, 1986), and avian species (Fenlon, 1985). Dairy products seem the most susceptible to contamination due to contaminated raw milk (Kampelmacher, 1963). Poultry and meats can be contaminated through slaughter, processing, packaging, and distribution. Surveys of retail, oven-ready poultry in the United Kingdom revealed that

14.7% (Gitter, 1976) and 60% (Pini and Gilbert, 1988) of samples evaluated were contaminated with *L. monocytogenes*. The presence of *L. monocytogenes* in slaughterhouse effluents (Watkins and Sleath, 1981) further suggested that contamination of meat and poultry may be common. In Australia, *L. monocytogenes* was detected on 93 of 175 samples (53%) of vacuum-packed processed meats obtained from retail stores (Grau and Vanderlinde, 1992). *L. monocytogenes* can grow and accumulate in dairy products (Rosenow and Marth, 1987) and meats (McClain and Lee, 1988), and can compete with other microflora once it is present in foods.

The infection of humans usually occurs orally, such as with contaminated food, or possibly by direct contact with infected animals. High incidence of carriers among ruminants may result from *L. monocytogenes* existing as a saprophytic organism, which lives naturally in a plant-soil environment and can therefore easily be contracted by grazing animals. Dairy farm sources include fecal or silage contamination. Danish pigs are raised in closed houses and fed dry, pelletized feeds. The pigs are normally not fed silage or fresh vegetables, thus there are few opportunities for contamination by ingesting decayed vegetable material in nature. As a consequence of these feeding habits, only 1.7% of the pigs were *L. monocytogenes* excretors (Skovgaard and Nørrung, 1989). These authors found 63% of minced pork samples were contaminated by *Listeria* spp., and *L. monocytogenes* were found in 12% of the samples. Only 4.1% of the pig feces samples were found positive for *Listeria* spp. Compartmentalization of *L. monocytogenes* inside muscle fibers may explain its presence in the muscle and meat (Johnson et al, 1990). On the other hand, fecal excretion of *L. monocytogenes* is very common in cattle, where levels of a few percentages to over 50% have been reported (Skovgaard and Morgen, 1988). In the study by Skovgaard and Morgen (1988), *L. monocytogenes* was found in 67% of the wet feed samples and 52% of the cattle feces

were contaminated. It was concluded that fecal contamination along the slaughter line may be a leading cause of the dissemination of *Listeria* spp., independent of the number of fecal excretors.

Importance   *Listeria* is not a new organism. Prior to the 1980s, outbreaks of *Listeria monocytogenes* infections were associated with abortions and encephalitis in sheep and cattle caused by the ingestion of spoiled silage and were primarily of veterinary concern (Lovett and Twedt, 1988). In recent years, convenient, microwave-ready, refrigerated foods have become very popular, thus more concern has developed for the survival and growth of this organism in these food items. Foodborne illness may occur by *L. monocytogenes* if inadequate heating is applied before serving because it can survive at refrigeration temperatures.

Incubation time for the listeriosis to develop is 1 day to a few weeks after ingestion. At first, mild "flu-like" symptoms will occur. Following this stage, virulent forms may multiply in the intestinal tract, be absorbed in the blood, and transported to other vital organs. The manifestations of listeriosis include septicemia (which in pregnant women can lead to abortion or stillbirth), endocarditis, pneumonia, conjunctivitis, pharyngitis, cutaneous papules, pustules, urethritis, and meningitis. The organism possesses an emerging threat to public health, especially people such as pregnant women, infants, elderly, alcoholics, and immunocompromised, AIDS patients (Johnson et al., 1990).

Survival and growth of *Listeria* spp. on meats   Chilled and refrigerated foods are especially susceptible to higher populations of *L. monocytogenes* due to its ability to proliferate at refrigeration temperatures (Brackett, 1988). Hence, refrigeration, which traditionally has been an important means of controlling the growth of foodborne pathogens, is not an effective measure for controlling *Listeria* spp.; this is

an important factor in *Listeria*'s significance in the food chain (Lovett and Twedt, 1988). On the other hand, *L. monocytogenes* prefers to grow in a 5% O_2/10% CO_2 atmosphere. Therefore, some modified atmospheres may generally reduce microbial load but survival of this pathogen needs to be monitored.

     L. monocytogenes is a psychrotroph that will not grow well at room temperature (25°C) or below 0°C. Junttila et al. (1988) reported that the minimum growth temperature of *L. monocytogenes* was 1.1°C. *L. monocytogenes* can survive, but not grow, in ground beef at 4°C for 2 weeks; package permeability to oxygen did not affect survival (Johnson et al., 1988). Growth of this organism on the vacuum-packaged beef depended on the temperature of storage, the pH of the lean, and the type of tissue. Growth was more rapid at 5.3°C than at 0°C, and on meat of pH 6.0-6.1 rather than pH 5.5-5.7. Growth occurred earlier (a shorter lag phase) on the fat, and the population of *L. monocytogenes* was higher on fatty tissue than on lean muscle tissue as time extended (Grau and Vanderlinde, 1990). This confirmed a study by Gouet et al. (1978), who found that the growth of *L. monocytogenes* was possible in meat with a pH >6.0, and meats having high initial pH (e.g., DFD beef) could pose a hazard. Aerobic growth models of *L. monocytogenes* on beef lean (effect of pH and temperature) and fatty tissue (effect of temperature) were formulated with two modified Arrhenius equations by Grau and Vanderlinde (1993).

     L. monocytogenes is quite tolerant to sodium chloride. This organism can survive for more than 18 weeks in trypticase soy broth containing 25.5% NaCl and incubated at 4°C; but less survival was reported when incubation temperatures of 22 or 37°C were used (Shahamat et al., 1980). Junttila et al. (1989) increased the salt content of Finnish fermented sausages from 3 to 3.5% NaCl and found little effect on *L. monocytogenes*.

Evidence for growth of *L. monocytogenes* in cooked meat is less substantial due to its heat sensitivity. However, USDA-FSIS (USDA, 1989b) established a zero tolerance level of *L. monocytogenes* in cooked ready-to-eat meat products, increasing the need to reduce the incidence of *L. monocytogenes* in precooked meats. Hardin et al. (1993) stressed that the inhibition during postpasteurization depended on the heating temperature and time used in the process. *L. monocytogenes* was inoculated in beef roasts, at a level of 10^9 cells per bag, followed by packaging in cook-in bags and pasteurization. The results showed heating at 96°C for 5 min eliminated the *L. monocytogenes* population and maintained lower counts (from initial inoculum) during storage at both 4°C and 10°C. Temperature, pH, and sodium chloride played important roles to the growth of *L. monocytogenes* (McClure et al., 1991). Glass and Doyle (1989) stressed that the rate of growth *L. monocytogenes* depended largely upon the type of product and the pH of the product. This study investigated the fate of *L. monocytogenes* during refrigerated storage (4.4°C) by monitoring 8 kinds of processed meat products. They concluded that growth was most prolific on processed poultry products; the organism generally grew well on meats near or above pH 6 and poorly or not at all on products near or below pH 5.

There is no definite correlation between the number of *L. monocytogenes* present on ground meat and aerobic plate counts (Karches and Teufel, 1988). A correlation may exist between the presence of *Listeria* and high levels of other foodborne pathogens in raw and ready-to-eat meat products. Le Guilloux et al. (1980) indicated meat samples which were contaminated with *Listeria* spp. also had large amount of staphylococci, coliforms, *Clostridium perfringens*, and salmonellae.

Irradiation effects on *L. monocytogenes*　　　The D_{10} values for *L. monocytogenes* have been determined in various environments. Patterson (1989)

inoculated four strains of *L. monocytogenes* in phosphate-buffered saline and in minced poultry meat; D_{10} values of 0.318-0.494 kGy and 0.417-0.553 kGy were obtained individually. Radomyski et al. (1993) summarized many experiments and found that the D_{10} values of *Listeria* were 0.20-1.03 kGy, usually at 0.4-0.6 kGy. Huhtanen et al. (1989) concluded that 2.0 kGy would be sufficient to destroy 4 log of *Listeria*. However, a later study (Mead et al., 1990) found that injured cells will grow during storage and Patterson et al. (1993) concluded that in poultry meat, 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 and so their growth should not be a problem during storage.

Appropriate irradiation (2.0-3.0 kGy) should inhibit or kill *L. monocytogenes*, thus ensure the food safety and extend the shelf-life. Shelf-life of fresh, vacuum-packaged pork loins, treated with 3.0 kGy irradiation and stored at 2-4°C, were examined (Lebepe, 1990). This dose extended the microbiological shelf-life to more than 90 days compared with 41 days of nonirradiated loins. However, two irradiated samples throughout experimental period at 2-4°C tested positive for *L. monocytogenes*. They suggested that more than 3 kGy may be required to kill all *Listeria*.

Atmosphere is critical to *L. monocytogenes* growth. Chickens inoculated with *L. monocytogenes* were irradiated at a dose of 2.5 kGy and during subsequent storage at 4°C for 15 days, the cells were recovered only from vacuum packaged chickens after 7 days of storage. No *L. monocytogenes* was detected in irradiated samples which were stored under air. In nonirradiated chickens, *L. monocytogenes* proliferated similarly in both air- and vacuum-packaged chickens (Varabioff et al., 1992).

In the study by Huhtanen et al. (1989), *Listeria* spp. cultures which were centrifuged and resuspended in solutions containing organic materials were more

resistant to radiation than those resuspended in water. It was thought that free radicals formed after irradiation, which exert a damaging "indirect" effect on cellular constituents, can be scavenged by organic molecules particularly those containing sulfhydryl groups.

Salmonella spp.

Characteristics   *Salmonella* is a genus of the family Enterobacteriaceae characterized as Gram-negative rods, producing acid and gas from dextrose and maltose and generally not fermenting lactose or sucrose. Most species are motile and facultative anaerobes.

Since salmonellae are pathogenic, they generally are considered to be mesophilic, with optimal growth temperature at 35-37°C. However, some strains appear to be psychrotrophic. The lowest temperatures at which growth has been reported are 5.3°C for *S. heidelberg* and 6.2°C for *S. typhimurium* (Matches and Liston, 1968). Temperatures of around 45°C are the upper limit for growth. These organisms can grow at pH from 4 to 9 and water activity (A_W) of 0.93 to 0.96.

The classification of *Salmonella* spp. by antigenic analysis is based upon the Kauffmann-White Scheme. This scheme uses both somatic (O) and flagellar (H) antigens. The K antigens are capsular antigens that lie at the periphery of the cell and prevent access of anti-O agglutinins (antibodies) to their homologous somatic antigens. New species or serotypes are continually being found.

Source   Most foodborne salmonellosis outbreaks are associated with consumption of foods of animal origin, including eggs, milk, poultry, and red meat products (Bryan, 1988). In a nationwide Canadian survey of meat samples at slaughter houses in 1983-1986, *Salmonella spp.* were isolated from turkey, broiler chicken, pork, beef, and veal at 69.1%, 60.9%, 17.5%, 2.6%, and 4.1% of carcasses (Lammerding et

al., 1988). Feces were the major source of *Salmonella spp*. (Mafu et al., 1989). Contamination of meat usually occurs during postmortem processing or handling, with transfer of pathogens from skin, feathers, hair, or intestinal contents to equipment and carcasses (Stolle, 1987). Carcass sanitizing with use of high pressure water spray with or without chlorine added, detergents, or organic acid solution may decrease microbial loads as well as some of the pathogens on carcass surfaces (Dickson and Anderson, 1992). Benedict et al. (1991) reported that organisms like *Salmonella* spp. appear to attach preferentially to connective tissue fibers, rather than to myofibrils. Muscle fiber swelling and shrinkage during processing permits some microbial entrapment between muscle bundles.

Occasionally non-animal products are also found to be contaminated with the *Salmonella* organism. This usually occurs through cross-contamination with ingredients of animal origin.

Importance   Salmonellosis is an infection caused by the action of the organism in the intestine. Numbers of cells on the order of 10^7-10^9/g of food are generally necessary for symptoms to develop. The usual incubation period is reported as 6 to 48 hr (CDC, 1983). The most common reported symptom is diarrhea, followed by abdominal cramps, fever, nausea, vomiting, chills, and headache. Duration of gastroenteritis usually lasts for two to three days, but if further infection occurs, the illness may persist for months or years, and it may result in death. Children are more susceptible than adults to prolonged excretion of the organism (Banwart, 1989).

Some serotypes of *Salmonella* are more virulent than others. *Salmonella typhi* is the most virulent member of this group and is responsible for typhoid fever. *Salmonella dublin* has recently been termed a life-threatening hazard by the FDA due to mortality of infected individuals of about 25% versus 0.5% for other types. However, it is generally

accepted that any serotype of *Salmonella* is potentially hazardous for man and other warm-blooded animals. Salmonellosis is a substantial illness in the U.S., having the greatest cost among the bacterial foodborne diseases (Bean et al., 1990). It is estimated that there is an annual incidence of almost 2-4 million cases of salmonellosis in the U.S., which results in as many as 2,000 deaths per year. According to Todd's (1989) estimation, about $1 to $4 billion are lost each year due to unsalable food products, product recalls, medical expenses, and lack of consumer confidence in food safety.

Subtherapeutic doses of antibiotics have been used intensively as additives to animal feeds for prophylaxis or to enhance the rate of feed conversion. The major disadvantage is development of antibiotic-resistant strains of Enterobacteriaceae and this characteristic can be disseminated by R-factors on plasmids of the bacterial cell, including *Salmonella* spp. Resistance to antibiotics enhances pathogenicity and virulence to humans and animals (Cohen and Tauxe, 1986).

Survival and growth of _Salmonella_ on meats The interaction of other microorganisms naturally present in the food with salmonellae can result in the inhibition of these potential pathogens. Many studies have shown inhibition of *Salmonella* by lactic acid bacteria. Gilliland and Speck (1972) believed that antagonistic action was caused by factors in addition to the acidic environment created by bacterial fermentation. Vacuum packaging has been shown to extend the shelf-life of fresh meat (Fu et al., 1992). This packaging method inhibits the growth of most Gram negative, spoilage microorganisms thus lactic flora become predominant. Such products are generally safe and free of foodborne pathogens with the possible exception of *Y. enterocolitica* and *S. aureus*. The effectiveness of inhibition by lactic flora is highly temperature dependent, with temperatures at or below 0°C showing the greatest effect (Mead, 1983). *Salmonella* spp.

will not compete well with other organisms under vacuum packaging and constant low refrigeration temperatures.

Unlike *Staphylococcus aureus*, *Salmonella* spp. are unable to tolerate high salt concentrations. The growth of *Salmonella* is generally inhibited in the presence of 3-4% NaCl. Increased inhibition was reported in ground pork stored for 14 days at 10°C when the NaCl concentration was varied from 0 to 3.5%; no growth was detectable at 5% NaCl (Alford and Palumbo, 1969). These authors also found that higher temperatures facilitated initiation of growth in medium of greater salinity, but high concentrations of NaCl protracted the lag period and decreased the rate of growth. The bacteriostatic effect of NaCl also has been described in terms of brine concentration. This value defines the salt concentration in the water phase as the salt content relative to the sum of the salt added and the water content of the food. Brine concentrations of 5.3% in meat are inhibitory to *Salmonella* (Prost and Riemann, 1967); while above 9% brine is reported to be bactericidal (Jay, 1986c).

Irradiation effects on *Salmonella* Application of irradiation on poultry has been approved by USDA, with doses of 1.5-3.0 kGy (USDA, 1992b). Poultry is easily contaminated during processing and appropriate irradiation is an efficient way to eliminate food pathogens. *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* were the top three pathogens accounted for in over 4 million cases of illness; 96 %, 100%, and 85% of these cases were caused by food, individually, and at least half attributable to meat and poultry (USDA, 1993). The D_{10} values of *Salmonella* were reported to range from 0.20 to 1.29 kGy, with most at 0.4-0.7 kGy (Radomyski et al., 1993). Therefore, the legal dose of irradiation on poultry products should eliminate at least 2 log of Salmonellae.

Katta et al. (1991) indicated that gamma irradiation of broiler chickens at doses ranging from 1.5 to 2.0 kGy eliminated 99% of the microbial load, without adversely affecting sensory and nutritional values. From Varabioff et al. (1992), a dose of 2.5 kGy decreased total counts about 3.6 log from inoculated 8 log CFU/g. No significant difference was observed between chickens packaged in air or vacuum at 0, 4, and 7 days of storage at 4°C. Kahan and Howker (1978) irradiated fresh eviscerated broiler chickens, and concluded that a combination of 2.5 kGy and storage at 1.6°C extended shelf-life to 15 days without deleterious effects on color, odor, or taste.

Effects of heat and ionizing radiation on *Salmonella typhimurium* in chicken meat were evaluated by Thayer et al. (1991). They found that heating inoculated chicken meat before irradiation did not sensitize the bacteria to the radiation, while treating the inoculated chicken meat with gamma radiation made the *Salmonella* much more sensitive to the effects of heat.

Yersinia enterocolitica

Characteristics   *Y. enterocolitica* is characterized by Gram negative, coccoid-shaped rods (0.99-3.54 μm x 0.52-1.27 μm). It is a facultative anaerobic bacterium and belongs to the genus *Yersinia* in the Enterobacteriaceae family. For most strains, the growth temperature range in nutrient broth is 1 to 44°C, with the optimal growth temperature for *Y. enterocolitica* is 22-25°C, which is slightly lower than most other Enterobacteriaceae. The pH range for the survival and growth of *Y. enterocolitica* is 4.6-9.0, with optimum range being pH 7.0-8.0 (Hanna et al., 1977a). When incubated at 4°C, it grew slowly at pH 5.2-5.4, and showed active growth at pH 5.6-7.6 (Seelye and Yearbury, 1979).

Food may contain both environmental (avirulent) and potentially pathogenic varieties. Both types of strains are most often motile at room temperature and nonmotile at 37°C, yield an acid-slant, acid-butt, no-gas, and no-H_2S on TSI test, and are urease-positive and phenylalanine deaminase-negative. They are also typically arginine-negative, lysine-negative, and ornithine-positive (Stern, 1982).

Importance   *Y. enterocolitica* presents a very special problem as a foodborne pathogen because it is a psychrotroph. Gastroenteritis is by far the most frequently encountered manifestation. Acute non-complicated enteritis is observed most often in children below age 7. Severe abdominal pain, one of the symptoms of human yersiniosis, has been misdiagnosed as appendicitis, and unnecessary appendectomies have been performed as a result (CDC, 1982). Its invasive type of infection is similar to shigellosis and causes gastroenteritis with diarrhea, fever, abdominal pain, and vomiting (Doyle, 1988). Some strains of *Y. enterocolitica* produce an enterotoxin similar to *E. coli* ST, which stimulates the activity of cyclic GMP (Inoue et al., 1983).

Y. enterocolitica has been classified into approximately 60 serogroups on the basis of O antigens (Wauters, 1981). Non of so-called '*Y. enterocolitica*-like organisms' have been associated with human or animal disease, with the exception of a few atypical cases. All strains capable of causing yersiniosis belong to *Y. enterocolitica*. The most important causative agents in man are O:3, O:5,27, O:8, and O:9. Distinctive geographical distributions of the serotypes exist but there is no reasonable explanation for this. The O:3 strains are predominant throughout the world. In the U.S. the O:8 serotype is most frequently incriminated, but seldom found outside the political boundaries of the U.S. and western Canada. The O:3 and O:9 strains are rarely observed within the U.S. (Stern, 1982).

Source   Varieties of *Y. enterocolitica* isolated from the environment are fairly

common in nonchlorinated drinking water, unpasteurized milk, fresh vegetables, raw

shellfish, and raw or rare meats. Fortunately, only certain virulent strains are involved in

human infection.

This organism has been isolated from almost every type of domestic livestock.

Dogs, cats, and rats may occasionally be fecal carriers of O:3 and O:9. The relative

intimate contact between man and pets suggests a potential reciprocal transmission,

although such an epidemiological link has not yet been clearly confirmed (Kapperud,

1991). Swine are considered a major natural reservoir for the serotypes of *Y.*

enterocolitica which occur in human diseases (De Boer and Nouws, 1991). A study in

Belgium (Tauxe et al., 1987) showed that 58% of gastroenteritis caused from *Y.*

enterocolitica was strongly associated with eating raw pork. Nesbakken et al. (1987)

indicated that porcine and human isolates harbor virulent plasmids (40-50 MDa) with

identical restriction patterns, providing additional support for attributing importance to the

role of pigs in the epidemiology of human infection. The apparent rarity of yersiniosis in

Moslem countries also supports the potential role of pork as a vehicle of *Y. enterocolitica*

infection (Kapperud, 1991).

This organism has been isolated from many locations on pigs, but mostly from

the oral cavity (Nesbakken, 1988), including the tongue (Doyle, 1981), throat

(Schiemann and Fleming, 1981), and tonsils (Hanna et al., 1980). Enterotoxigenic

strains were found in minced meat (Kleinlein and Untermann, 1990), various retail pork

products (Schiemann, 1980), and vacuum-packages of beef and lamb (Hanna et al.,

1976). Fecal contamination, however, is the most important source on meat and meat

products (Andersen, 1988; Mafu et al., 1989). Cross-contamination by food handlers or

food containers allows multiplication during refrigerated storage. If these foods are

consumed without being cooked or reheated to temperatures that destroy this pathogen, virulent strains will cause infection (Snyder and Poland, 1990; 1991).

Nesbakken (1988) concluded that modern slaughtering technology and the routines followed by meat inspection personnel have probably contributed to the relatively high rates of these bacteria on carcass cut surfaces. Thus, the introduction of changes in slaughtering technology and meat inspection practices presently being employed in Norwegian slaughterhouses was suggested. The use of a mechanized bung cutter, connected with enclosing the anus and rectum in a plastic bag to minimize fecal contamination, was found to reduce the rate of contamination (Andersen, 1988). However, direct transmission from pigs to humans has not been clearly demonstrated. Pork appears to be an important vehicle, but no outbreak or human case of yersiniosis has been directly linked to pork handling or consumption (Schiemann, 1989).

Survival and growth of *Yersinia* on meats   If contaminated ingredients are used to make a product, followed by storage at refrigeration temperatures, *Yersinia* spp. can survive. Therefore, it is considered to be an emerging pathogen for human beings. *Y. enterocolitica* has been shown to grow on raw beef held at 0-1°C (Hanna et al., 1977b), on high pH beef at as low as –2°C (Gill and Reichel, 1989) and minced beef at 1 and 4°C (Kleinlein and Untermann, 1990); it can also survive in frozen foods for long periods (Schiemann, 1989). When ground beef and fresh sausage were inoculated with *Y. enterocolitica*, the bacteria grew in samples at 25°C (up to 48 hours of storage), 4°C (1-7 days), 0°C (0-2 days), and –20°C (1-5 weeks) (Moustafa et al., 1988). It can grow at pH 4.6 and in a salt concentration up to 7%, and survive in raw and cooked meat products even when they are vacuum-packed (Mitchell and Varabioff, 1988).

The ability of *Y. enterocolitica* to compete with other psychrotrophs normally present in foods may be poor (Schiemann, 1989). Cooked beef samples were inoculated

with *Y. enterocolitica*, *L. monocytogenes*, and *Aeromonas hydrophila* and incubated at 5 and 10°C under both aerobic and vacuum-packaged (anaerobic) conditions. Results showed growth rates were similar for *L. monocytogenes* and *A. hydrophila*, but *Y. enterocolitica* grew more slowly under anaerobic conditions. Therefore, vacuum packaging is no guaranteed safeguard against the growth of these cold-tolerant pathogens on cooked beef, even if good temperature control is maintained (Hudson and Mott, 1993).

It has been suggested that DFD (Dark Firm and Dry) meat may favor the growth of *Y. enterocolitica*, not only because of the elevated pH level, but also because of the low sugar content, a factor which may result in reduced competition with the lactobacillus flora (Skjelkvålé, 1981).

While the contamination of pork with *Y. enterocolitica* cannot totally be avoided, its survival and growth in processed meat products could be found. Raevuori et al. (1978) inoculated a sausage mass with *Y. enterocolitica* at the level of 5.23 log cells/g. Sodium nitrite was added at 0, 50, 80, 100, and 120 mg/kg levels and three starter cultures were used. Less than 2 log of *Y. enterocolitica* O:3 could still be detected in fermented sausages at day 35 when 0 or 50 mg/kg sodium nitrite was added, with a pH range of 4.9-5.4. But when the concentration of 150 mg/kg sodium nitrite was added can decrease the population of *Y. enterocolitica* from 5-6 log CFU/g to less than 2 log CFU/g. Adding 3% sodium chloride and 156 mg/kg sodium nitrite to heated meat may achieve better inhibition of *Y. enterocolitica* O:3 than the lactic acid bacteria alone (Raccach and Henningen, 1984).

Irradiation effects on *Yersinia* The D_{10} values of *Yersinia* spp. were 0.04-0.21 kGy (Radomyski et al., 1993). This is lower than *Salmonella* spp. or *Listeria* spp., thus irradiation applied to inhibit *Salmonella* spp. or *Listeria* spp. should also

eliminate *Yersinia* spp. Microbial isolates were identified from ground pork irradiated at 1 kGy (Ehioba et al., 1988) and showed that a small percentage of *Yersinia* spp. were detected at 12 days of storage at 5°C. The meat did not reach the predefined spoilage level (10^7 CFU/g) until after 11 days at 5°C. This organism was also detected in nonirradiated samples at the same time, even in meat already spoiled at day 9. Tarkowski et al. (1984b) indicated that doses as low as 1 kGy were effective in reducing *Y. enterocolitica* by more than 4 log on raw beef. However, the organism showed a 2 log cycle growth on some samples after 7 days of storage at 3°C.

Chicken carcasses were dipped in fermented whey, lactic acid or water, and irradiated at 2.5 kGy (Lamuka et al., 1992). The results showed that irradiation significantly reduced *Yersinia* spp. to an undetectable level (3.03 log decrease). However, some injured cells recovered and multiplied as evidenced by continuous growth with time in irradiated samples.

Escherichia coli O157:H7

Characteristics *E. coli* is classified in the family Enterobacteriaceae and its taxonomic features include Gram negative, nonsporeforming, straight rods that may be peritrichously flagellated or nonmotile. The organism is a facultative anaerobe capable of using simple sugars and minimal basal media for its growth.

Important biochemical tests (IMViC) used for identifying *E. coli* include indole production (I), methyl red (M), Voges-Proskauer (Vi), and utilization of citrate (C) as the sole source of carbon. About 95% of *E. coli* have the IMViC pattern + + – – and are classified as biotype 1, other *E. coli* have the IMViC pattern – + – – and are biotype 2. Another classification is the serotyping scheme, which was based on *E. coli* somatic (O), flagellar (H), and capsular (K) antigens.

   E. coli O157:H7 are typical of most *E. coli*, with some exceptions. *E. coli* O157:H7 is unable to grow well, if at all, at 44-45.5°C. Normal procedures used for detecting fecal coliforms and subsequently *E. coli*, which use incubation temperatures at 44-45.5°C, will not isolate *E. coli* O157:H7. Raghubeer and Matches (1990) indicated that the temperature range for *E. coli* O157:H7 growth and gas production in EC medium within 48 hr was 19.3 to 41.0°C. Growth with no gas formation was observed at 16.4 and 42.5°C.

   Data from Conner (1992) indicate that *E. coli* O157:H7 is similar in salt tolerance to other serotypes of *E. coli*. This organism was more sensitive to NaCl at 10°C than at 37°C. At ≥8% NaCl, the inhibition of growth was bactericidal. At ≥6% NaCl at 10°C, the effect was only bacteriostatic and became ineffective upon increasing incubation temperature to 37°C.

   Differences in biochemical properties include sorbitol fermentation, ß-glucuronidase activity, and production of enterohemolysin (Doyle, 1991). More than 90% of *E. coli* isolates of human origin ferment sorbitol within 24 hr, but *E. coli* O157:H7 does not (Ratnam et al., 1988). More than 90% of *E. coli* possess the enzyme ß-glucuronidase which is the basis for a rapid fluorogenic assay for *E. coli* (Feng and Hartman, 1982). In this assay, a fluorescent product is produced and easily detected by the reaction of ß-glucuronidase with added substrate 4-methyl-umbelliferone glucuronide (MUG). *E. coli* O157:H7 is MUG assay negative, indicating that ß-glucuronidase activity is not phenotypically expressed by this organism (Doyle and Schoeni, 1984). A type of hemolysin, called enterohemolysin, has been found by Beutin et al. (1989), which is common to verotoxin-producing *E. coli* but not other *E. coli* strains. They determined that most (32 of 33) verotoxic *E. coli* O157:H7 or O157:H⁻ isolates produced enterohemolysin.

E. coli O157:H7 has no unusual heat resistance and can survive well in ground beef during frozen storage for up to 9 months (Doyle and Schoeni, 1984). Although CO_2 inhibits the growth of some Gram negative bacteria (Silliker and Wolfe, 1980), no significant effect of modified atmospheres (5% to 10% CO_2) on *E. coli* O157:H7 (Hao and Brackett, 1993) has been observed. Results also indicated that this organism could survive but not grow at 4°C, even with modified atmospheres. Higher CO_2 concentration, greater than 10%, may have some inhibitory effect.

Importance   Non-virulent strains of *E. coli* are always present in the human intestinal tract and could be used as an indicator organism for fecal contamination. Coliforms in general and *E. coli* in particular have been used for this purpose. So far, there have been four virulent strains of *E. coli* found (Olsvik et al., 1991). Enteropathogenic *E. coli* (EPEC) has been implicated in outbreaks of infantile and neonatal diarrhea mainly from non-food origin. Another strain called enteroinvasive *E. coli* (EIEC) produces severe dysentery similar to shigellosis. A third strain, enterotoxigenic *E. coli* (ETEC) can produce toxins after ingestion by humans, and is mainly associated with diarrhea in developing countries. This strain is most commonly associated with traveler's diarrhea in the U.S.

Enterohemorrhagic *E. coli* (EHEC), the fourth and most virulent strain, is of current concern because it may cause hemorrhagic colitis, with symptoms of bloody diarrhea, severe abdominal pain, and cramps. This organism can also cause hemolytic uremic syndrome (HUS), the leading cause of kidney failure in children, as well as damage to the central nervous system. Another manifestation is thrombotic thrombocytopenic purpura (TTP), which often develops blood clots in the brain; death usually results. The incubation period for this organism ranges from 3 to 9 days, with a median of 4 days, and the duration of illness ranges from 2 to 9 days, with a median of 2

days. The disease is distinguished from the dysentery described in shigellosis or invasive *E. coli* gastroenteritis by the lack of fever and by the bloody discharge resembling lower gastrointestinal bleeding (Padhye and Doyle, 1992). Dupont et al. (1971) determined, on the basis of a human study, that ingestion of 10^6 to 10^8 cells of pathogenic strains of *E. coli* were needed to cause illness in healthy individuals. However, a much lower number of microorganisms can cause illness in young children, the elderly and immune compromised people. Both studies (Cimolai et al., 1990; Rowe et al., 1991) showed that females had a greater risk of developing hemolytic anemia and HUS after infection with *E. coli* O157:H7 than did males.

The latest outbreak was in January 1993, in which more than 475 people became seriously ill and eventually three children died, after they ate hamburgers at Jack-in-the-Box fast food stores in Washington, Idaho, California, and Nevada. This outbreak resulted in wide spread publicity and efforts by researchers to detect and control this deadly microorganism to prevent further outbreaks. The culprit of this outbreak was *E. coli* O157:H7 which survived in undercooked hamburger. FDA is now recommending that hamburgers be cooked to a minimum internal temperature of 68.3°C (155°F) instead of 60°C (140°F) (Mermelstein, 1993). In fact, before the outbreak, Shipp et al. (1992) had indicated that beef patties cooked at endpoint temperature 60°C were found to be unsafe, and recommended cooking to an endpoint temperature of 66°C for the appropriate holding time to result in a microbiologically safe product.

Thermal inactivation studies of *E. coli* O157:H7 in ground beef have revealed that the organism has no unusual heat resistance, with D_{10} values of 270, 45, 24, and 9.6 sec at 57.2, 60.0, 62.8, and 64.3°C, respectively (Doyle and Schoeni, 1984). This organism is more heat sensitive than typical isolates of salmonellae. D'Aoust et al. (1988) also showed that pasteurization of milk (72°C, 16.2 sec) will kill more than 10^4

E. coli O157:H7 per ml. Therefore, well-cooked meat and the pasteurized milk should ensure safety. This organism can survive well in ground beef during frozen storage (Doyle and Schoeni, 1984). The population of *E. coli* O157:H7 did not change significantly in ground beef frozen as patties at –80°C and held at –20°C for up to 9 months .

Source   *E. coli* O157:H7 was first identified as a cause of human illness in 1982 when it was associated with two food-related outbreaks of hemorrhagic colitis in the states of Oregon (26 cases) and Michigan (21 cases) (Riley et al., 1983). Since then, many outbreaks of *E. coli* O157:H7 infections have been reported in the U.S., Canada, and United Kingdom (Doyle, 1991). Foods of animal origin, principally from dairy cattle and their products, milk and beef were associated with infections, but the organism also has been found in water and apple cider. Young calves within herds are more likely than adult cattle to harbor the organism, but even in calves the prevalence is very low (Mermelstein, 1993). The organism can also be isolated from the feces of asymptomatic cattle (Montenegro et al., 1990; Wells et al., 1991) and from raw milk (Wells et al., 1991). Although more than half of the outbreaks of *E. coli* O157:H7 infections have been attributed to ground beef, little is known about risk factors for sporadic cases.

According to a survey by Doyle and Schoeni (1987) on retail meats from grocery stores in Madison, WI., approximately 2% of beef, poultry, pork, and lamb samples were contaminated, whereas meats from Calgary, Alberta, Canada, stores yielded significantly higher levels of contamination. Other foods such as turkey sandwiches have also been associated with infections (Carter et al., 1987), but the source was not identified. It is likely due to cross-contamination with raw beef during food preparation contamination by infected food handlers. Water was implicated as the vehicle of a community outbreak in Missouri (Doyle, 1991). McGowan et al. (1989) isolated *E. coli*

O157:H7 from a countryside reservoir, thus providing further evidence that this organism can be waterborne.

Survival and growth of _E. coli_ O157:H7 on meats   A commercial fermented sausage batter was inoculated with 4.68 log of _E. coli_ O157:H7 per gram. The organism survived but did not grow during fermentation (pH 4.8), drying, or subsequent storage at 4°C, and decreased by about 2 log CFU/g after 2 months of storage (Glass et al., 1992). Thus these authors suggested that it is incumbent on manufacturers of fermented sausage to use raw meat that contains no or very few _E. coli_ O157:H7.

"Heat shock" studies on _E. coli_ O157:H7 were conducted by Collins and Murano (1993). Ground beef and pork inoculated with _E. coli_ O157:H7 were heated to achieve a target temperature of 55°C at rates of 0.8, 1.0, or 5.0°C/min. Results indicated that meat samples exposed to the slowest heat rate before processing resulted in the highest number of survivors after a 55°C heat treatment, thus the heat processing rate can affect the survival of this pathogen when exposed to heat.

Irradiation effects on _E. coli_ O157:H7   A study of the effects of irradiation dose (0 to 2.0 kGy), temperature (−20 to 20°C), and atmosphere (air and vacuum) on _E. coli_ O157:H7 in mechanically deboned chicken meat was conducted by Thayer and Boyd (1993). Differences in irradiation dose and temperature significantly affected the results. They found that 90% of the viable _E. coli_ in chicken meat was eliminated by doses of 0.27 kGy at 5°C and 0.42 kGy at −5°C. They also inoculated _E. coli_ O157:H7 in ground beef. Unlike nonirradiated samples, no measurable verotoxin was detected in ground beef which had been inoculated with $10^{4.8}$ CFU/g, irradiated at a minimum dose of 1.5 kGy, and temperature abused at 35°C for 20 hr.

CHAPTER HI. EFFECT OF IRRADIATION ON SURVIVAL OF SELECTED PATHOGENS AND ON QUALITY ATTRIBUTES OF COOKED PORK CHOPS AND CURED HAM

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Abstract

Listeria monocytogenes and *Salmonella typhimurium* were inoculated in cooked pork chops (pumped with salt/polyphosphate brine or untreated) and cured hams. The samples were irradiated at low (0.75 to 0.90 kGy) or medium doses (1.8 to 2.0 kGy), with each dose being delivered at either a low (8.2 ft/sec) or high (17.9 ft/sec) dose-rate. After irradiation the samples were stored at 7°C for 7 days, followed by 2 days at 25°C. Low dose irradiation reduced *L. monocytogenes* by more than 2 log, and *S. typhimurium* by 1 to 3 log. Pathogen counts and total plate counts (TPC) in uninoculated samples were reduced to undetectable levels by the medium doses. No quality attributes (pH, lipid oxidation, color, and sensory color and odor) were affected even at medium doses. No dose-rate or brine effect was observed. Effect of nitrite in reducing both pathogens and TPC during 7°C storage was significant ($P < 0.05$) in ham, especially when combined with low dose irradiation.

Introduction

Changes in processing and packaging technology have produced many new and convenient food products. Precooked meat has been used widely and is very popular among consumers for the convenience it provides. It is expected that precooked meat in a vacuum package would have an extended shelf-life, since mild cooking decreases the

initial microbial counts and vacuum packaging inhibits spoilage microorganisms and minimizes lipid oxidation (Jones et al., 1987; Stites et al., 1989).

Safety of such "microwave-ready" products has been considered. The potential *Clostridium botulinum* hazards associated with extended shelf-life refrigerated foods were reviewed by Conner et al. (1989). Sodium nitrite is usually used to prevent this risk in cured meat. However, addition of nitrite can affect meat color, so it is not suitable for cooked, uncured product. Precooked, vacuum-packaged, refrigerated meat products may also provide a potential growth environment for psychrotrophic organisms such as *Listeria monocytogenes* (Unda et al., 1991). This organism may survive and grow in atmospheres which are high in CO_2, such as in modified atmosphere or vacuum packaging. Evidence for the presence of *L. monocytogenes* in cooked meat is less substantial due to the sensitivity of this organism to heat. In Australia, however, *L. monocytogenes* was detected on 93 of 175 samples (53%) of vacuum-packed processed meats obtained from retail stores (Grau and Vanderlinde, 1992). Given the potential for growth of this organism, the USDA-FSIS (USDA, 1989) has established a zero tolerance level for *L. monocytogenes* in cooked ready-to-eat meat products. *Salmonella* spp. do not grow at temperatures greater than 47°C. Ideally, these organisms should not be found in cooked meat products. However, post-cooking contamination may occur during chilling and before packaging, thus posing a hazard. *Salmonella* spp. grow optimally at 30-45°C and can grow slowly at a temperature range of 5-15°C. However, evidence has also shown that this organism can proliferate in fresh meats at 2.0°C and on shell eggs at 4.0°C within 6 and 10 days, respectively (D'Aoust, 1991). Thus refrigeration temperature would not guarantee the safety of contaminated cooked meat.

Ham, a cured product, usually can be stored longer than fresh meat. Adequate curing and smoking/cooking provide for its extended shelf-life. Nitrite is used as a

coloring agent to give ham an attractive pink color and to suppress growth of *C. botulinum*. Salt and phosphate are usually added in processed meat to provide flavor and texture. They may inhibit growth of some microorganisms, depending on the concentration in the product, and on other factors such as pH and storage temperature (D'Aoust, 1991; McClure et al., 1991).

Irradiation provides a means to reduce the need for chemical additives, since it decreases the microbial load and eliminates food pathogens from foods. This process also decreases the opportunity of postprocessing contamination, if packaged before being irradiated. There have been some questions raised regarding the possible effect of irradiation dose-rate on destruction of microorganisms. It is suspected that a high dose-rate may not inhibit as many microorganisms as a low dose-rate due to recombination of radicals formed during irradiation at a high dose-rate, rather than reaction of the radicals with food components at a low dose-rate. Also, it is postulated that high dose-rate irradiation may result in creation of an anoxic environment, resulting in fewer oxygen radicals being formed. Very little work has been done on the comparative effect of different types of radiation on microorganisms by irradiating samples at the same dose-rate, because of technical limitations between these two different sources. However, some reports have shown no difference in the effect on microorganisms between high dose-rate and low dose-rate, as demonstrated by comparing gamma irradiation (low dose-rate) and electron irradiation (high dose-rate) (Hayashi, 1991). In our study, a single irradiation source (linear accelerator) and a fixed energy level were used. It is generally recognized that the energy level does not directly influence the antimicrobial effect of food irradiation. For example, Watanabe et al. (1988) reported that the energy (0.5-3.0 MeV) of electron beams did not influence the D_{10} value of *Bacillus pumilus* spores. Different dose-rates were adjusted by changing power level and conveyor speeds.

More research is required for "microwave-ready pork" and cured hams to demonstrate the advantages of irradiation, and to gain regulatory approval of this process. Little work has been done about dose-rate effects on pathogens present in meat products. The objectives of this study were: to evaluate the effects of low or medium doses of irradiation on survival of *S. typhimurium* and *L. monocytogenes* in microwave-ready chops (pumped or unpumped with salt/polyphosphate brine) and cured boneless ham; to determine the effect of dose-rate on survival of these organisms and other attributes after irradiation processing; and to monitor product attribute changes such as pH, lipid oxidation, color, and odor in these products after irradiation and during storage.

Materials and Methods

Sample preparation

Chops   Fresh boneless pork loins were purchased from a commercial source, directly from the processing line, and prepared at the Iowa State University Meat Laboratory. Eighty lb of brine solution, containing 8 lb of salt and 3.2 lb of polyphosphate, was prepared. Six loins ($\approx$72 lb) were weighed and pumped with brine to 110% weight by using a Townsend Model 1400 injector (Townsend Engineering Inc., Des Moines, IA) to achieve approximately 1.0% salt and 0.4% polyphosphate in the loins. The other six loins were used as unpumped controls. After pumping, all loins were cooked (without smoked) at 80°C of smokehouse temperature, in an Alkar smokehouse (Alkar, Lodi, WI) to an internal temperature 67°C. Cooking end point was determined by using a thermocouple inserted in the geometric center of the thickest loin. Loins were chilled at 2-4°C for 2 hr and sliced into 2.5-cm (1-in) thick chops.

Hams   Hams needed for three replications were made at the same batch. Frozen boneless pork hams were thawed at 2-4°C, then weighed ($\approx$75 lb) and pumped

with brine to 125% of green weight by using a Townsend Model 1400 injector (Townsend Engineering Inc., Des Moines, IA). The curing solution (100 lb) was composed of water (80.2 lb), salt (11 lb), sugar (6.6 lb), polyphosphate (2.2 lb), erythorbate (99.8 g), and sodium nitrite (28.3 g), with the concentration in the final product being 2.5%, 1.5%, 0.5%, 500 ppm, and 140 ppm, respectively. The hams were tumbled, stuffed, and cooked in an Alkar smokehouse (Alkar, Lodi, WI) with preset cooking program. The six-step cooking program was: (1) hot air, at 82°C for 45 min, (2) drying, at 82 °C for 15 min, (3) hot smoke, with hickory sawdust at 82°C for 45 min, (4) hot smoke, with hickory sawdust at 82°C, 74% moisture for 1 hr, (5) hot air, at 82°C, until core temperature at 63°C, (6) cooking and scalding, at 85°C, until core temperature at 68°C. Then chilled at 2-4°C for at least 72 hr. Hams were removed from casings and sliced into 5-mm thick slices by using a slicer.

External inoculation and packaging

Cooked chops from pumped and unpumped loins, and ham slices were individually divided into three subgroups for inoculation with *Listeria monocytogenes* or *Salmonella typhimurium*, and uninoculated samples were used as controls.

Listeria monocytogenes Scott A was grown in trypticase soy broth containing 0.6% yeast extract (TSB+YE) at 35°C for 24 hr until the stationary phase was reached. The culture was transferred to a fresh broth and incubated for 6 hr to the log phase (as determined by previous growth curve experiments). A 2-ml inoculum was transferred to a 10-ml TSB+YE broth and diluted in a 1200-ml 0.1% peptone water, final concentration approximately 7 log cells/ml. Using the same procedure, *Salmonella typhimurium* was grown in trypticase soy broth (TSB) and approximately 6 log cells/ml was obtained.

For surface inoculation, chops or hams were dipped in the inoculum solution for 10 min and drained on sterilized racks for 2 min. Final cell concentrations were

approximately 6 log and 5 log cells of *L. monocytogenes* and *S. typhimurium*, respectively. Samples were placed in high-barrier packaging pouches having O_2 permeability of <2.5 cm^3/645 cm^2/24 hr at 23°C and 0% RH and water vapor transmission of <1.0 g/645 cm^2/24 hr at 38°C and 90% RH (Curlon™ 863 Saran, Curwood Inc. New London, WI). Vacuum packaging was completed using a model A300 CVP machine (CVP System, Inc., Downers Grove, IL). After packaging, all samples were placed in cardboard boxes and stored at 2-4°C for 12 hr before being irradiated.

Irradiation

Samples were irradiated at the Linear Accelerator Facility at ISU using ambient temperature (≈25°C). Chops and hams were irradiated separately because of difference in density and thickness of these two products. Each inoculated or uninoculated group was further divided into five subgroups and assigned to different irradiation processing treatments. For chops, samples were irradiated at 0.75 kGy or 2.0 kGy, each at low (10.8 or 4.3 ft/sec) or high (25.8 or 8.8 ft/sec) dose-rates, with non-irradiated samples used as controls. Low or high dose-rates were produced by varying the power level (1 or 3 kW) and conveyor speed (ft/sec, short or long exposure time) in the irradiator. Actual absorbed doses were measured by placing alanine pellets on both sides of a chop. An Electron Paramagnetic Resonance (EPR) instrument was used to determine the absorbed dose (Bruker Instruments, Inc., Billerica, MA), which was calibrated according to the national standards established by the National Institute of Standards and Technology (NIST).

Ham slices were irradiated at 0.90 kGy or 1.8 kGy, each at low (12.0 or 5.7 ft/sec) and high (27.0 or 12.0 ft/sec) dose-rates. Actual absorbed doses were measured by placing GAF chromic films on both sides of a ham slice. The film is nylon based,

with a thin layer of radiochromic film intertwined. Radiation activated color changing was measured by using a FWT 100 reader (Farwest Technologies, Inc., Goleta, CA), which was calibrated with data in EPR instrument.

All combinations of treatments for chops and hams were analyzed immediately after irradiation (day 0). Samples were also stored at 7°C for 7 days in order to simulate consumer storage, followed by temperature abuse at 25°C for two days to simulate mishandling of these products after purchase. Preliminary studies have shown that storage of processed meats at 2-4°C (simulate wholesale/retail storage) increased TPC by only approximately 1.0 log CFU/g, therefore 7°C was directly used in this study to simulate temperature of household refrigerator.

Physical and chemical analyses

Measurements of pH, lipid oxidation (TBA), and color were conducted on uninoculated samples except for those products where the total plate counts showed obvious spoilage ($>10^7$ CFU/g), or when obvious off-odor developed. Sample pH was measured by using a slurry of 10-g of meat with 100-ml distilled water, a pH meter (Fisher Accumet Model 925, Fisher Scientific, Pittsburgh, PA) and a sealed combination electrode with silver/silver chloride reference (Omega Engineering, Inc., Stamford, CT). Measurement of thiobarbituric acid (TBA) values, an index of lipid oxidation, was performed by the method of Tarladgis et al. (1960). Duplicate readings were recorded for each sample for pH and TBA tests. Color (L, a, and b) measurements were made with a HunterLab Labscan spectrophotometer (Hunter Associated Laboratories Inc., Reston, VA). An average value was obtained from three random locations on each sample surface.

Microbiological analyses

Packages from each treatment were aseptically opened on sampling days and a 25-g sample was removed using a sterile scalpel. The samples were homogenized

separately for 2 min in a sterile bag containing 225-ml of sterile 0.1% peptone water in a Stomacher lab blender (Model 400; Tekmer™ Co., Cincinnati, OH). Each sampling solution (1-ml) was added to tubes containing 10 ml enrichment broth to determine the presence of bacteria that might be too low to be enumerated by direct plating. Listeria enrichment broth (UVM, DIFCO) and Selenite broth (SB, DIFCO) were used for *L. monocytogenes* and *S. typhimurium*, respectively.

Serial dilutions were prepared according to recommended microbiological procedures (Vanderzant and Splittstoesser, 1992), surface plated onto Plate Count Agar (PCA, DIFCO), and incubated aerobically at 25°C for 48 hr to enumerate total plate counts. *L. monocytogenes* was enumerated by plating onto Modified Oxford agar (MOX, DIFCO) containing Colistin sulfate-Moxalactam antimicrobic supplement (DIFCO), followed by incubation at 35°C for 48 hr. Typical *Listeria* colonies surrounded by a black zone were identified by Gram stain, catalase, oxidase, tumbling motility at 25°C, and confirmed by Micro-ID Listeria (Organon Teknika, Durham, NC). *S. typhimurium* was enumerated by plating on xylose-lysine-desoxycholate agar (XLD, DIFCO), followed by incubation at 35°C for 48 hr. Presumptive black colonies were identified by using triple sugar iron (TSI) slants, and confirmed by using Enterotube® II (ROCHE Diagnostic Systems, Montclair, NJ). Plates were counted with a Darkfield Quebec colony counter (American Optical Co., New York, NY).

Sensory color and odor evaluation

Only uninoculated samples were used for color and odor evaluation which was conducted at day 0. A 10-member sensory panel evaluated samples by using a 5-point descriptive scale for surface color (1 = dark gray; 5 = red) and odor (1 = pleasant, no off-odor; 5 = extreme off-odor). Chops or hams were temperature tempered at room

temperature for 2 hr, unwrapped, and placed in ziploc pouches for evaluation. Ten chops or five ham samples were evaluated at each session.

Statistical analyses

The experiment of cooked chops included 30 treatment combinations; two pump treatments, three inoculation treatments, and five irradiation treatments (2 x 3 x 5 = 30). Measurements of pH, TBA, Hunter color L, a, b and sensory evaluation were only conducted on uninoculated samples; thus both included 10 (2 x 1 x 5) treatment combinations. Microbiological, physical and chemical analyses were conducted at day 0, 7, and 9; while sensory evaluation only conducted at day 0. Three replications were conducted. Total number of chops used was 390 [(30 x 3 + 10 x 3 + 10 x 1) x 3].

The experiment of hams included 15 treatment combinations; three inoculation treatments, and five irradiation treatments (3 x 5 = 15). The same sampling design as chops, therefore the total number of hams used was 195.

Microbiological data were transformed into logarithms of the number of Colony Forming Unit/g ($\log_{10}$ CFU/g). Average data and standard errors were calculated from three replications. Different sampling times (day 0, 7, and 9) were analyzed separately because different temperatures were used for storage. Physical, chemical, microbiological, and sensory data were analyzed by using the Statistical Analysis System (SAS Institute Inc., 1986). The analysis of variance (ANOVA) procedure was used to detect the significance of replications, pumping (chops only), dose, and pumping by dose (chops only); dose-rate and other combination effects were also evaluated. If no significant difference existed between low and high dose-rates, data were averaged for analyses. Comparisons of means were based on Duncan's multiple range test.

Results and Discussion

Effects of dose-rate on microorganisms and quality attributes

No dose-rate effect was observed for the range of dose-rate used in this study (data not shown). Technically, using a single radiation source means that dose-rate differences that can be obtained are relatively limited. There was more than a two fold difference between the low and high dose-rates used. However the dose-rates may not have been significantly different to make a difference in the numbers of survivors to irradiation. Radiation generated by electron beams from electron accelerators is categorized as a high dose-rate treatment. This treatment has low penetrating ability but it is fairly rapid. Further investigation on the effect of dose-rate on survival of microorganisms should be conducted using dose-rate that differ by a wider margin than those used here. It is suggested that conversion of electron-beam to X-ray mode, in which there is a loss of energy, could be used to achieve very low dose-rates.

Since no significant difference caused by two dose-rates used in this study, data were averaged and analyzed.

Effects of irradiation on microorganisms

Listeria monocytogenes Irradiation was effective in reducing *L. monocytogenes* in inoculated chops or hams. For chops, irradiation at 0.75 kGy had a significant effect ($P<0.05$) in reducing 2 log CFU/g; after storage for 7 days, the number of *L. monocytogenes* cells was not significantly different ($P>0.05$) from controls; further temperature abuse allowed *L. monocytogenes* to proliferate to 8 log CFU/g, the same as controls (Fig. 1). Low dose (0.9 kGy) irradiation was more effective on hams than chops in reducing *L. monocytogenes* (Fig. 2). *L. monocytogenes* was reduced by 3 log CFU/g in hams (Fig. 2), compared with a 2 log CFU/g reduction in chops (Fig. 1). This effect was maintained after 7 days of storage, with the number of cells not exceeding 4

log CFU/g (Fig. 2). However, the cells grew after temperature abuse, at 25°C, reaching the same number in both ham and chops, of approximately 8 log CFU/g.

Medium dose irradiation resulted in an even greater reduction in the number of survivors of *L. monocytogenes*. Results from Fig. 1 and Fig. 2 show that almost all cells in either chops or hams were eliminated to undetectable levels after irradiation at 2.0 kGy. However, this dose only injured *L. monocytogenes*, since the cells were able to recover when the temperature was elevated to 25°C. Even so, the final counts in both products were lower than those in controls or in samples exposed to low dose irradiation.

No brine effect was observed in the chops immediately after irradiation, or after extended storage (Fig. 1). Concentrations of salt and polyphosphate in pumped chops were 1.0 and 0.4%, respectively. This salt concentration was not high enough to affect growth of *L. monocytogenes*. This is not surprising, since this organism can survive in trypticase soy broth containing 25.5% NaCl and incubated at 4°C for more than 18 weeks (Shahamat et al., 1980). Junttila et al. (1989) determined that salt content of Finnish fermented sausages at 3 to 3.5% had little effect on *L. monocytogenes*. Although a higher concentration may have inhibited more spoilage organisms, it would also increase the risk of selecting for *Staphylococcus aureus* and contribute to the development of undesired flavor. Polyphosphate was added in the brine solution to increase water holding capacity, improve color retention, flavor, and cooking yield of meat, and to chelate trace metal ions to retard development of rancidity (Pearson and Tauber, 1984). Polyphosphate could increase the pH, depending on the concentration used. However, pH values were not affected by brine concentration in pumped chops (Table 1). Temperature, pH, and sodium chloride played important roles in the growth of *L. monocytogenes* (McClure et al., 1991). From a study on processed meat products, Glass and Doyle (1989) suggested that this organism generally grows well on meats near

or above pH 6 and poorly or not at all on products near or below pH 5. In our study, the range of pH values of the samples was 5.5-6.0, with most of them being 5.7-5.9 (Table 1). A lack of change in pH during storage could explain why no significant difference (P>0.05) was detected in growth of *L. monocytogenes* between unpumped and pumped samples.

The number of *L. monocytogenes* increased by only 1 log after incubation at 7°C, in both non-irradiated and irradiated (0.9 kGy) hams. This suggests that this temperature somewhat inhibited the growth. Lower temperature is usually beneficial for this organism, since it can survive in ground beef at 4°C for 2 weeks (Johnson et al., 1988) and at temperature as low as 1.1°C (Junttila et al., 1988). In the review by Radomyski et al. (1993), results from an experiment with artificially inoculated samples were summarized. These indicated that the D_{10} value of *Listeria* spp. is usually between 0.4-0.6 kGy. From this information, medium doses (2.0 or 1.8 kGy) used in our study should have reduced the counts by 5 log CFU/g at most. However, reduction by almost 6 log CFU/g was observed instead. It has been suggested that vacuum packaging and refrigeration can enhance the inhibitory effect of irradiation. Comparing hams with pumped chops, a higher salt concentration (2.5% vs. 1.0%) and addition of nitrite in ham was probably responsible for the greater degree of inhibition of *L. monocytogenes*, especially when low dose irradiation was applied. Buchanan et al. (1989) concluded that nitrite can have significant bacteriostatic effect on *L. monocytogenes*, particularly if used in conjunction with low pH, vacuum packaging, high NaCl concentrations and adequate refrigeration.

Salmonella typhimurium Irradiation was also effective in reducing *S. typhimurium* in inoculated chops or hams. Low dose (0.75 or 0.90 kGy) irradiation reduced the numbers by 1 log in chops, and by 3 log in hams (Fig. 3 and Fig. 4). Unlike

L. monocytogenes, the numbers of *S. typhimurium* survivors did not increase after 7 days of storage at 7°C. *S. typhimurium* is inhibited effectively by refrigeration temperature because this organism is a mesophile, preferring room temperature over refrigeration. *S. typhimurium* has comparable D_{10} value (0.4-0.7 kGy) to *L. monocytogenes* (0.4-0.6 kGy) (Radomyski et al., 1993), thus medium doses like those used in our study (2.0 or 1.8 kGy) reduced cell counts to undetectable levels in both products. Combined with refrigeration at 7°C, no *S. typhimurium* was detected even after 7 days of storage. Since these medium doses only injured *S. typhimurium*, survivors were still able to proliferate when the temperature was elevated to 25°C.

There was no significant (P>0.05) effect on *S. typhimurium* caused by brine injection in chops. Higher salt concentration and addition of nitrite in hams enhanced inhibition of *S. typhimurium* during storage, especially when low dose irradiation was used. After storage at 7°C for 7 days, low dose irradiation decreased *S. typhimurium* in hams close to undetectable levels (Fig. 4), while 1-3 log CFU/g of this organism was found in chops at the same storage time (Fig. 3).

Uninoculated samples   No *L. monocytogenes* or *S. typhimurium* survivors were detected in uninoculated chops or hams, indicating that precooking did eliminate both pathogens, if they were present in the products before packaging. Precooking "microwave-ready" chops provided for good microbiological quality of these products, which was evidenced by low (2 log CFU/g) initial TPC. Low dose (0.75 kGy) irradiation showed no significant difference (P>0.05) in TPC compared with controls, but a significant (P<0.05) effect in reducing TPC was seen during storage at 7°C (Fig. 5). No reduction was seen in unpumped samples after temperature abuse, while a 2 log reduction was observed in pumped samples. This inhibition may have been due to the brine injection. Thus, brine injection, used primarily for moisture retention and flavor,

seemed to provide some assistance for extending shelf-life, when combined with low-dose irradiation and refrigeration. Medium dose irradiation at 2.0 kGy decreased TPC to undetectable levels, which was significantly lower ($P<0.05$) than controls (non-irradiated). Irradiation at this level showed an effect during extended storage especially after temperature abuse, with samples irradiated at 2.0 kGy showing a decrease in total counts of 5 and 6 log CFU/g in unpumped and pumped chops compared with controls.

Ham is a processed meat product, which has been cured with salt, phosphate, nitrite and cooked, thus a low initial TPC (2.5 log CFU/g) was expected (Fig. 6). The combined treatment of vacuum packaging and refrigeration temperature (7°C) can further inhibit cell growth. When low (0.9 kGy) or medium (1.8 kGy) doses irradiation were applied, all organisms were reduced to undetectable levels even after storage at 7°C for 7 days. However, when storage temperature was elevated to 25°C, injured cells were still able to grow.

It has been identified that most microbial flora in vacuum packaging and/or irradiated samples are lactic acid bacteria (Lebepe et al., 1990). Although these bacteria are not pathogens, a high TPC is still considered an index of quality deterioration of meat products. Therefore, refrigeration is required to have a long shelf-life of these products even when irradiation is applied at low to medium doses.

Effects of irradiation on quality attributes

Irradiation did not affect pH, TBA, color and sensory color, odor attributes in chops or hams. Most of the pH values of chops and hams were about 5.7-5.9 (Table 1) and 6.2-6.3 (Table 2), indicating that pH was not affected by factors such as brine injection, curing agent, irradiation and storage condition in this study. The pH value is an index of water holding capacity and influences microbial growth. Consistent pH values throughout the storage period resulted in no such quality changes.

TBA value is an index of lipid oxidation, which is the major reason for the rancidity of meat and meat products. Pumped chops had similar (P>0.05) TBA values to unpumped ones at day 0 and 7 (Table 1). TBA values of most samples were within acceptable ranges (<1.00) indicating product stability in terms of lipid oxidation. Polyphosphate can chelate trace metal ions, which may help in retarding development of lipid oxidation in meats. Unda et al. (1991) showed 5 weeks of no change in lipid oxidation on microwave-ready beef roasts. Enzyme inhibition by pre-heating and exclusion of air are efficient in inhibiting lipid oxidation, resulting in lower TBA values, regardless of microbial quality. Most of the TBA values at day 9 were lower than at day 7, suggesting that microbial degradation of malonaldehyde and other thiobarbituric acid reactive substances (TBARS) occurring in meat samples may have caused lower TBA values (Moerck and Ball, 1974). Nitrite or a curing agent can also retard lipid oxidation. This was seen in all TBA values of hams at day 0, with values below 0.17 being observed (Table 2). These were even lower than those of pumped chops (0.32-0.47) (Table 1). In fact, TBA values remained below 0.5 throughout storage. No dose or dose-rate effect (P>0.05) was seen in TBA values, indicating that irradiation as high as 1.8 kGy would not increase this chemical reaction.

Color evaluation, conducted by machine and sensory panel, indicated no significant difference (P>0.05) between samples. For chops (Table 1), 'a' values (redness) of controls were smaller than those of irradiated samples at day 0 and 7, but this trend did not continue after temperature abuse. Color evaluation by panelists at day 0 did not detect much difference between treatments except that pumped chops had lowest value (Table 3). No significant change occurred on color 'L' and 'b' values. Nitrite is used in curing meat to improve color stability and inhibit growth of *Clostridium botulinum*. Nitrite is transformed into nitric oxide and combines with metmyoglobin,

after heating and smoking, to form a desirable pink pigment (nitroso-hemochrome). Because this pigment is relatively stable, surface color of ham was not changed by any level of irradiation. Hunter L, a, and b values (Table 2) and the color sensory panel (Table 4) showed no significant difference (P>0.05) between any samples.

The result of odor evaluation of chops showed that some irradiated samples emitted little off-odor compared with control, but this effect was not dose-dependent (Table 3). The predominant lactic acid bacteria within vacuum packaging may emit some "sour" odor. Whole sensory panel took about 45 min, including preparation, color and odor evaluation. It is possible that the odor may have dissipated in some unwrapped chops before they were evaluated by the panelists. Since these chops must be reheated before serving, any mild odor from irradiation could be masked by normal preparation procedures. No significant odor difference (P>0.05) in hams (Table 4) was detected by panelists.

Conclusions

Irradiation was effective in reducing *L. monocytogenes*, *S. typhimurium*, and total plate counts on "microwave-ready" chops and cured hams, especially at medium doses (1.8-2.0 kGy). Accompanied with refrigeration, irradiation should ensure the safety of these products, and should extend their shelf-life. Dose-rate was not found to have a significant effect on the organisms eunmerated. Irradiation at appropriate levels is also an effective means for decreasing the amount of salt or nitrite added in processed meat products.

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Table 1. Effects of irradiation on selected characteristics of unpumped (U), and pumped (P) cooked chops during storage at 7°C (day 0-7) and 25°C (day 7-9)

	Days of storage	Irradiation Dose (kGy)					
		0.0		0.75		2.0	
		U	P	U	P	U	P
pH	0	5.71^a	5.81^a	5.93^a	5.80^a	5.86^a	5.81^a
	7	5.92^a	5.86^a	5.84^a	5.85^a	5.88^a	5.85^a
	9	5.49^a	5.69^{bc}	5.57^c	5.88^{ab}	5.98^a	5.91^{ab}
TBA	0	0.57^{ab}	0.33^b	0.77^a	0.34^b	0.74^{ab}	0.42^{ab}
	7	1.81^a	0.43^b	1.68^a	0.39^b	1.27^{ab}	0.39^b
	9	0.42^a	0.38^a	0.48^a	0.34^a	0.45^a	0.35^a
L value	0	66.2^a	65.1^a	65.0^a	63.5^a	63.9^a	66.1^a
	7	65.2^a	65.1^a	63.2^a	65.3^a	65.8^a	62.4^a
	9	66.7^a	64.2^a	63.9^a	65.5^a	63.6^a	63.6^a
a value	0	2.7^c	2.9^c	3.4^{bc}	3.6^{ab}	4.3^a	4.3^a
	7	3.2^b	3.0^b	3.3^b	3.2^b	4.2^a	4.3^a
	9	4.0^{ab}	3.5^{ab}	4.1^a	3.2^b	3.6^{ab}	3.7^{ab}
b value	0	11.5^a	9.8^{bc}	11.0^a	9.7^c	10.4^b	9.2^d
	7	11.7^a	10.4^b	11.4^a	10.1^{bc}	10.9a^b	9.6^c
	9	11.1^a	9.8^b	10.9^a	10.2^b	11.1^a	10.1^b

$^{a-d}$Values within each row with the same superscripts are not significantly different (p>0.05).

Table 2. Effects of irradiation on selected characteristics of hams during storage at 7°C (day 0-7) and 25°C (day 7-9)

	Days of storage	Irradiation Dose (kGy)		
		0.0	0.90	1.8
pH	0	6.24[a]	6.26[a]	6.25[a]
	7	6.20[a]	6.23[a]	6.22[a]
	9	6.26[a]	6.34[a]	6.30[a]
TBA	0	0.15[b]	0.15[b]	0.17[a]
	7	0.48[a]	0.43[a]	0.43[a]
	9	0.39[a]	0.43[a]	0.35[a]
L value	0	56.2[a]	52.6[b]	53.9[ab]
	7	57.0[a]	54.9[a]	56.5[a]
	9	56.7[a]	55.4[a]	56.1[a]
a value	0	8.0[a]	7.9[a]	7.5[a]
	7	7.8[a]	8.1[a]	7.9[a]
	9	8.4[a]	8.7[a]	8.5[a]
b value	0	5.0[a]	4.0[a]	4.4[a]
	7	4.4[a]	4.1[a]	4.4[a]
	9	4.8[a]	4.8[a]	4.9[a]

[ab]Values within each row with the same superscripts are not significantly different (p>0.05).

Table 3. Effects of irradiation on sensory color and odor characteristics of
unpumped (U), and pumped (P) cooked chops

	Irradiation Dose (kGy)					
	0.0		0.75		2.0	
	U	P	U	P	U	P
color	2.4[a]	2.1[b]	2.6[a]	2.3[ab]	2.6[a]	2.6[a]
odor	1.6[d]	1.7[cd]	2.1[bc]	2.6[a]	2.6[a]	2.3[ab]

[a-d]Values within each row with the same superscripts are not significantly different
(p>0.05).

Table 4. Effects of irradiation on sensory color and
odor characteristics of hams

	Irradiation Dose (kGy)		
	0.0	0.90	1.8
color	4.0[a]	3.6[a]	3.9[a]
odor	3.4[a]	3.2[a]	3.5[a]

[a]Values within each row with the same superscripts are
not significantly different (p>0.05).
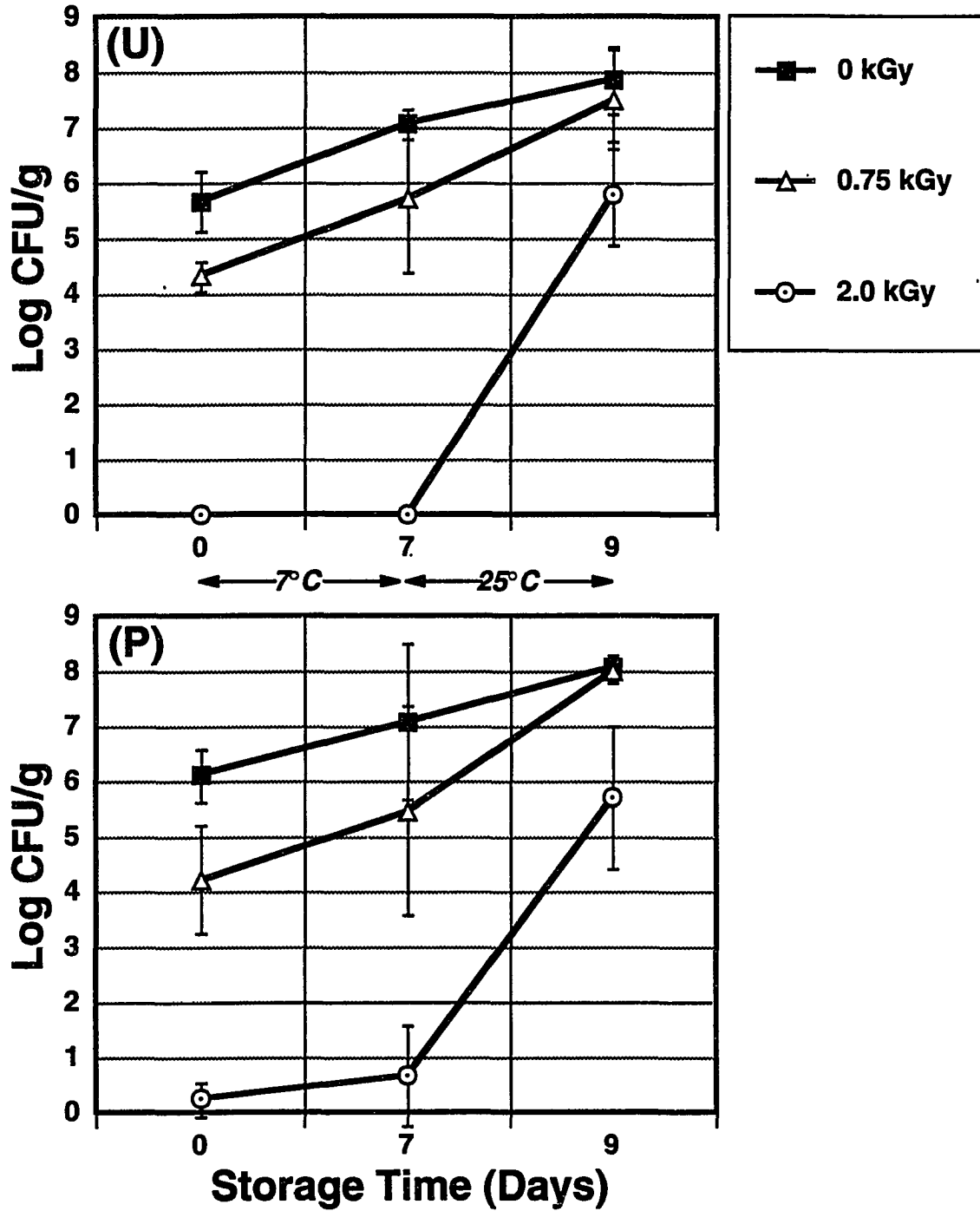
Figure 1. Growth of *L. monocytogenes* in unpumped (U) and pumped (P) cooked chops treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
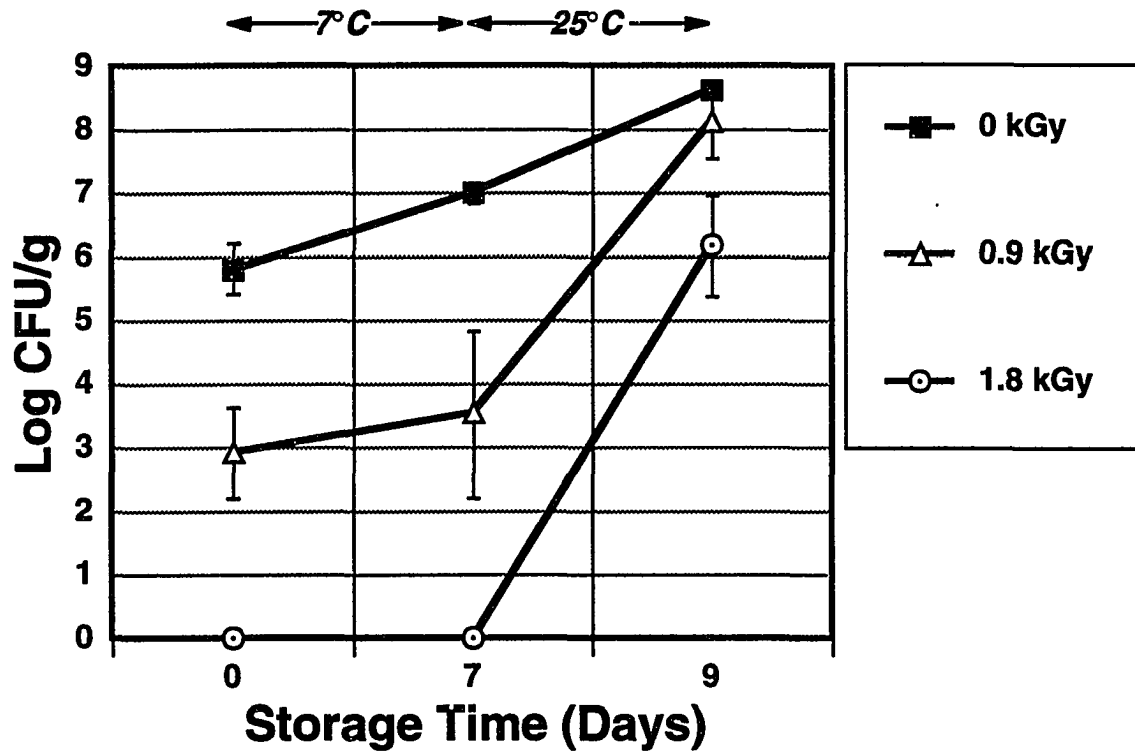
Figure 2. Growth of *L. monocytogenes* in hams treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
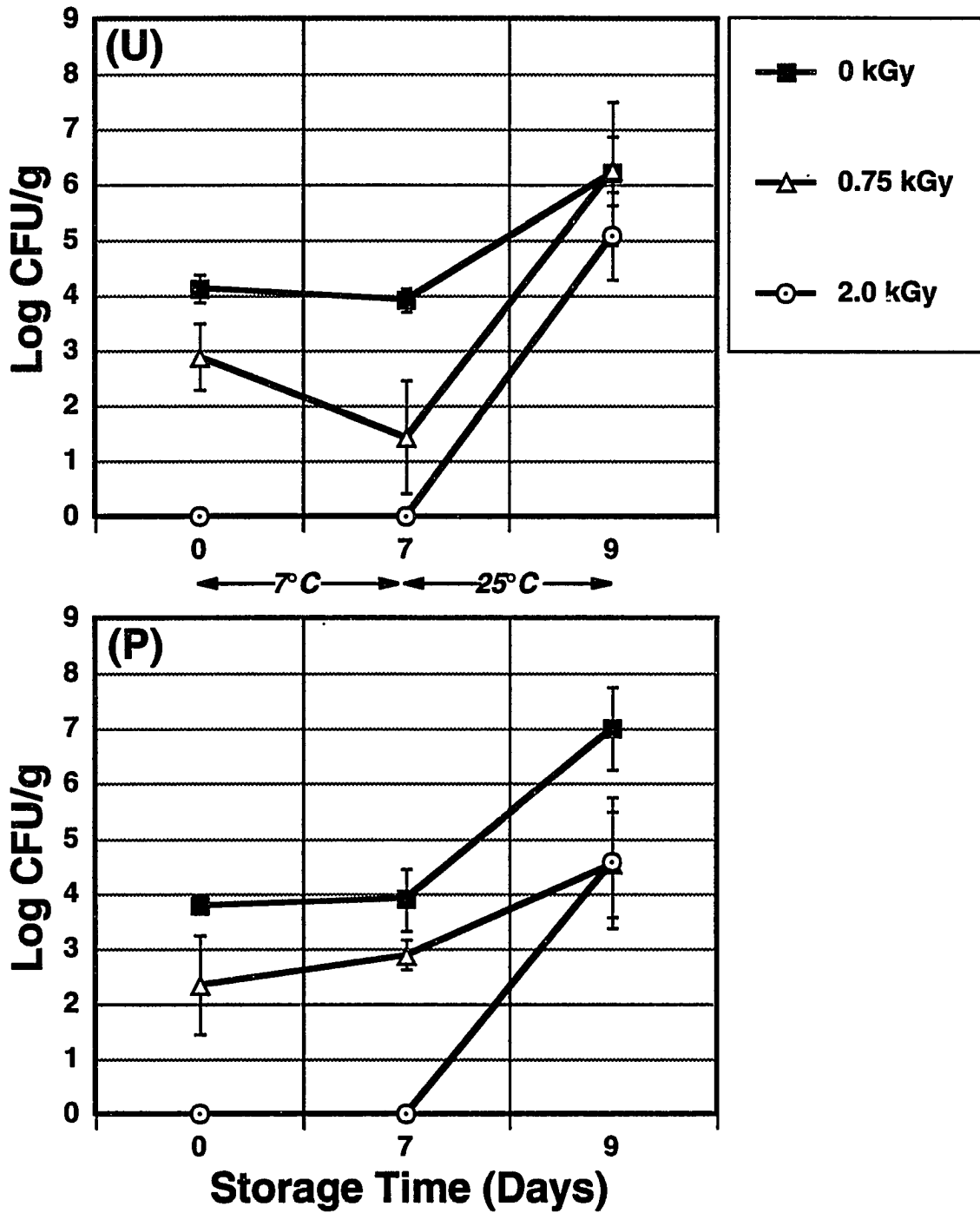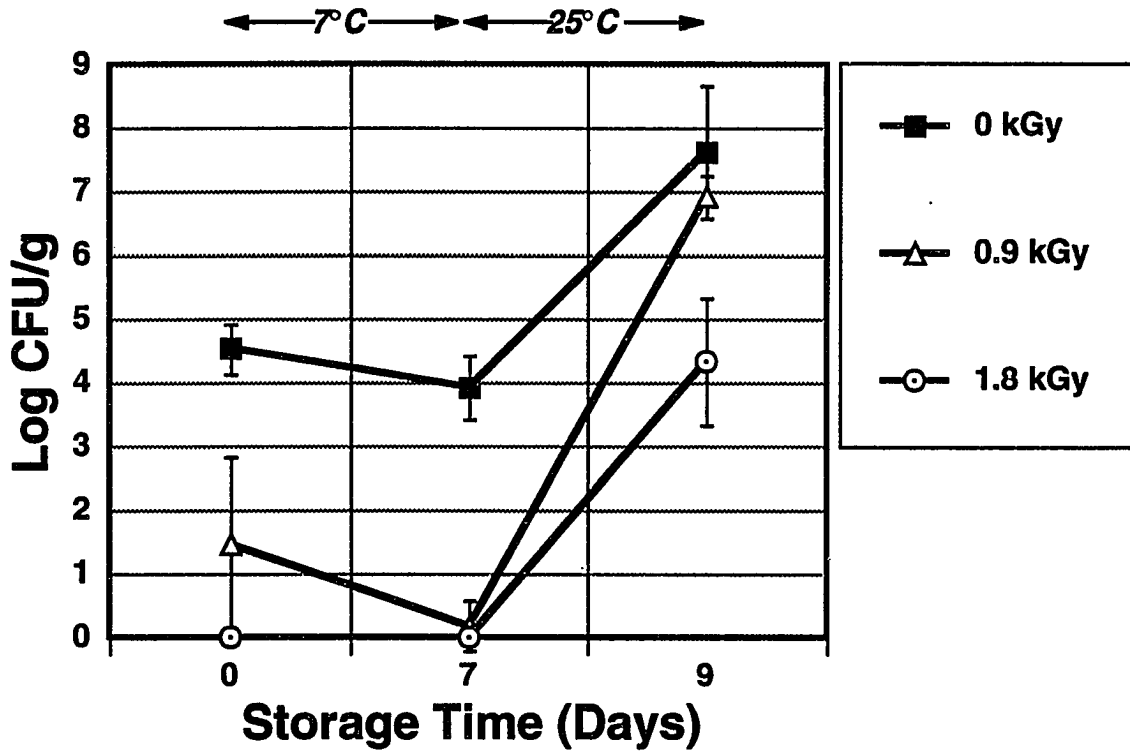
Figure 3. Growth of *S. typhimurium* in unpumped (U) and pumped (P) cooked chops treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication

Figure 4. Growth of *S. typhimurium* in hams treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
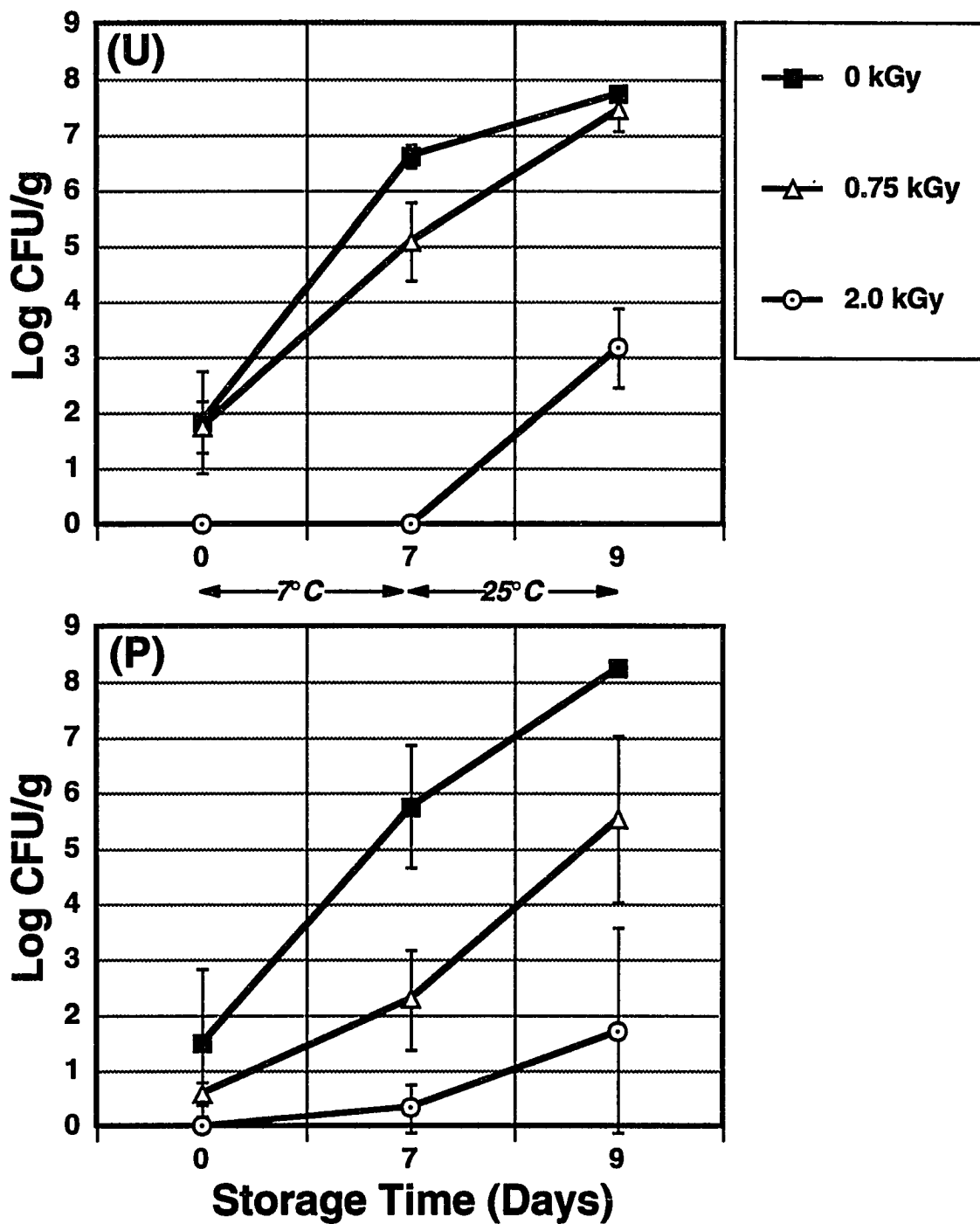
Figure 5. Total plate counts in unpumped (U) and pumped (P) cooked chops treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
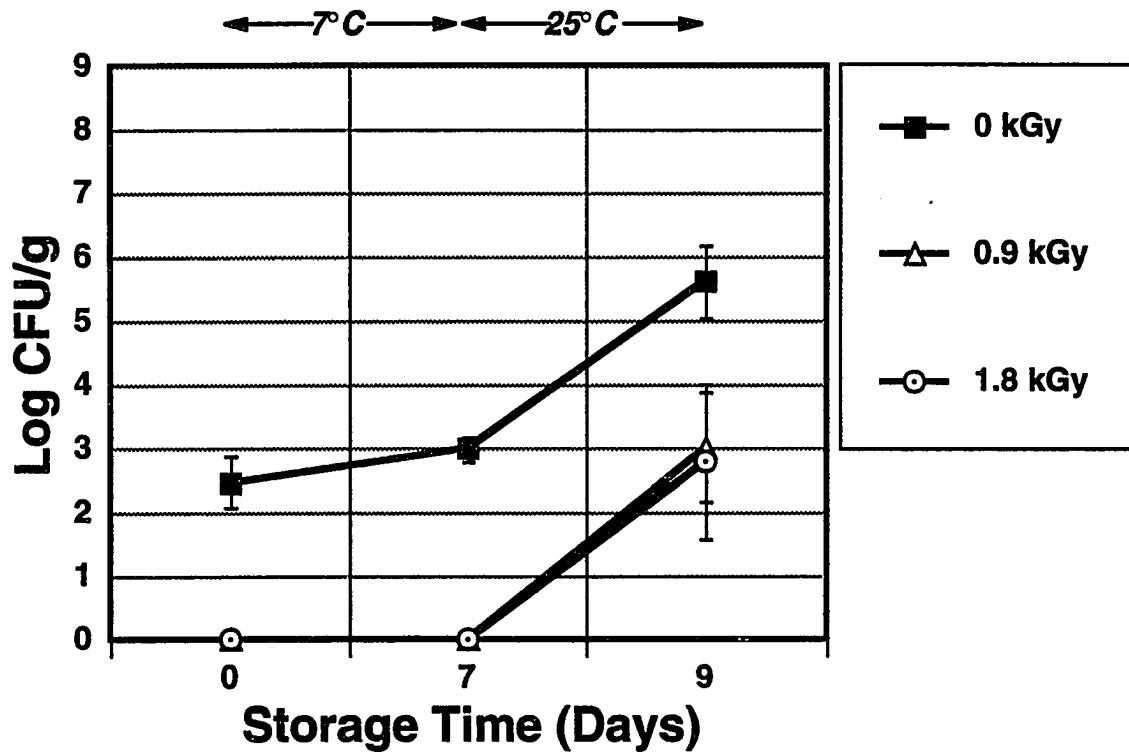
Figure 6. Total plate counts in hams treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication

CHAPTER IV. EFFECT OF IRRADIATION ON SURVIVAL OF SELECTED PATHOGENS AND ON QUALITY ATTRIBUTES OF BEEF LOINS, STEAKS, AND GROUND BEEF

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Abstract

Beef steaks and ground beef were inoculated with either *Listeria monocytogenes*, *Yersinia enterocolitica*, or *E. coli* O157:H7. Samples were packaged in air or under vacuum, and irradiated at low (0.60 to 0.80 kGy) or medium (1.5 to 2.0 kGy) doses, with each dose delivered at either a low (9.1 ft/sec) or high (22.8 ft/sec) dose-rate. After irradiation, the samples were stored at 7°C for 7 days, followed by 2 days at 25°C. The number of *L. monocytogenes* did not change during storage. Medium dose irradiation accompanied by 7°C storage resulted in no *Y. enterocolitica* or *E. coli* O157:H7 survivors being detected. There was no significant effect on survival of the pathogens to irradiation regardless of the dose-rate or storage atmosphere. No difference (P>0.05) was observed in pH or color in any of the samples. TBA values increased after 7 days of storage in all samples and a slight off-odor was detected in irradiated steaks as measured by sensory analysis.

Introduction

Food safety is a major concern of consumers and food processors. In January 1993, an outbreak of foodborne illness caused by consumption of hamburger contaminated with *E. coli* O157:H7 (Mermelstein, 1993) demonstrated how pathogens can contaminate meat and meat products, survive, grow, and cause disease when proper

processing is not applied. Although Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) system have been emphasized in the slaughtering and processing plants (Tompkin, 1983; Karr et al., 1994), sporadic contamination is still inevitable. Adequate storage and appropriate preparation by food service personnel and consumers is required to provide a defense against food pathogens.

There have been several reports on the contamination levels of *Listeria monocytogenes* (Johnson et al., 1990), *Yersinia enterocolitica* (Hanna et al., 1976; Myers et al., 1982), and *Escherichia coli* O157:H7 (Doyle and Schoeni, 1987) in beef. Since these organisms can survive refrigeration temperatures, the use of low temperature storage alone cannot be relied upon to keep meat safe from bacterial hazards (Palumbo, 1986). Irradiation provides an alternative in that it can decrease the microbial load of foods, and also eliminate specific pathogens on meat and meat products without changing their nutritive and sensory qualities (Niemand et al., 1981; Thayer et al., 1986; Skala et al., 1987; Thayer, 1993). The Food and Drug Administration (FDA) has approved the use of irradiation to reduce bacterial contamination of raw poultry (FDA, 1990), but no approval has been granted for beef. Research is required in order to demonstrate the benefits of irradiation in preventing outbreaks of foodborne illness from occurring in beef and beef products.

The effect of irradiation dose-rate on destruction of microorganisms is controversial. It is suspected that at a high dose-rate, recombination of radicals formed during irradiation takes precedence over reaction of radicals with food components, as occurs at a low dose-rate. This may increase the chances for microbial survival, due to a rapid depletion of toxic radicals in the environment. Also, it is postulated that high dose-rate irradiation may result in creation of an anoxic environment, resulting in fewer

oxygen radicals being formed. Very little work has been done on the comparative effect of irradiation on survival of microorganisms using different sources of radiation applied at the same dose-rate. However, some reports have shown no difference in the effect on microorganisms between high dose-rate and low dose-rate, as demonstrated by comparing gamma irradiation (low dose-rate) and electron irradiation (high dose-rate) (Hayashi, 1991). These differences, however, could be due to inherent differences in the source used, independent of dose-rate. It would be significant to determine whether there is an effect due to dose-rate by using the same source of irradiation.

Vacuum packaging has been using for primal and subprimal cuts since it can effectively inhibit growth of spoilage microorganisms in fresh meat. Refrigeration is also important in achieving this purpose. Gill and Newton (1978) indicated that storage of meat at temperatures between 1°C and 5°C can delay microbial spoilage. Most spoilage microorganisms in meat are Gram negative, with *Pseudomonas* being the most predominant, when meat is stored aerobically at refrigeration temperatures. Vacuum packaging will shift the microflora from Gram negative to Gram positive cells, thus delaying meat spoilage. Early studies have shown that lactic acid bacteria predominate in refrigerated vacuum-packaged pork (Lee et al., 1985) and beef (Beebe et al., 1976; Christopher et al., 1979). However, defects such as off-flavors, off-odors and undesirable colors after 8-11 weeks of refrigerated storage were reported by some studies (Pierson et al., 1970; Johnson, 1974) Vacuum packaging is often regarded as modified atmosphere in the sense that elevated levels (10-20%) of CO_2 are produced within vacuum packages by respiration of microorganisms and meat. Therefore, safety concerns on meat packaged under modified atmospheres need to be addressed in vacuum packaging of meat (Hintlian and Hotchkiss, 1986). Irradiation can provide another hurdle to ensure the safety of meat and meat products. However, modified atmosphere

packaging in combination with low dose irradiation (<10 kGy) may inhibit some spoilage microorganisms and thus result in a favored environment for surviving pathogens to proliferate. *Clostridium botulinum* has been shown to grow and produce toxin after low dose irradiation (Lambert et al., 1991). The objectives of this study were to evaluate the effect of low and medium doses of irradiation on survival of *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Escherichia coli* O157:H7 in beef loins, steaks and ground beef, to determine the effect of dose-rate on survival of these pathogens and quality attributes, and to monitor quality changes of pH, lipid oxidation, color and odor after irradiation at various doses and dose-rates.

Materials and Methods

Sample preparation

Ten vacuum-packed beef rib eye rolls were obtained directly from the processing line of a commercial source, and cut into 2.5-cm (1-in) thick steaks at the Iowa State University Meat Laboratory. Six lb of fresh ground beef (80% fat free) was obtained from a local supermarket, and a 25-g portion was weighed as a sampling unit.

External inoculation and packaging

Steaks and ground beef were individually divided into four groups for inoculation with *Listeria monocytogenes*, *Yersinia enterocolitica*, or *E. coli* O157:H7, and uninoculated samples were used as controls.

Listeria monocytogenes Scott A was grown in trypticase soy broth containing 0.6% yeast extract (TSB+YE) at 35°C for 24 hr to the stationary phase. The culture was then transferred to fresh broth and incubated for 6 hr to reach log phase (as determined by previous growth curve experiment). A 2-ml inoculum was transferred to a 10-ml TSB+YE broth and diluted in 1200-ml of 0.1% peptone water to final concentration

approximately 7 log cells/ml. By using the same procedure, *Y. enterocolitica* was grown in Brain Heart Infusion broth (BHI) at 25°C and approximately 7 log cells/ml was obtained; *E. coli* O157:H7 was grown in trypticase soy broth (TSB) at 35°C for 12 hr, transferred to fresh broth and incubated for 4 hr to reach log phase. After dilution, the final concentration of *E. coli* O157:H7 was approximately 7 log cells/ml.

Steaks were dipped in the inoculum solution for 10 min and drained on sterilized racks for 2 min. Inoculated and uninoculated treatment groups were individually divided into two subgroups, which were assigned to vacuum or air packaging. Steaks were placed in high-barrier pouches having O_2 permeability of <2.5 cm^3/645 cm^2/24 hr at 23°C and 0% RH and water vapor transmission of <1.0 g/645 cm^2/24 hr at 38°C and 90% RH (Curlon™ 863 Saran, Curwood Inc. New London, WI). Vacuum packaging was completed using a model A300 CVP machine (CVP System, Inc., Downers Grove, IL). Vacuum-packed steaks were used to simulate wholesale loins except for reduced size. Another subgroup of samples were placed in the same type of pouches sealed without vacuum. After packaging, all steaks were placed in cardboard boxes and stored at 2-4°C for 12 hr before being irradiated.

Each ground beef unit was inoculated with 1 ml of *Listeria monocytogenes*, *Yersinia enterocolitica*, or *E. coli* O157:H7 from prepared inoculum (1:100 dilution), and uninoculated samples used as a control set. Samples were wrapped in Saran Wrap™ (The Dow Chemical Co.) and were stored at 2-4°C for 12 hr before being irradiated.

Irradiation

Steaks were irradiated at the Linear Accelerator Facility at ISU using ambient temperature (≈25°C). Each vacuum or air subgroup was further divided into five groups and assigned to different irradiation processing treatments; doses used were 0.60 kGy and 1.5 kGy, each at low (12.6 and 5.9 ft/sec) and high (33.5 and 12.9 ft/sec) dose-rate,

with non-irradiated samples used as controls. Low or high dose-rate were produced by changing the power level (1 or 3 kW) and conveyor speed (ft/sec, short or long exposure time). Actual absorbed doses were measured by placing alanine pellets on both sides of a steak. An Electron Paramagnetic Resonance (EPR) instrument was used to determine the absorbed dose (Bruker Instruments, Inc., Billerica, MA), which was calibrated according to the national standards established by the National Institute of Standards and Technology (NIST).

The same protocol was used for treating ground beef, except that the actual doses were 0.80 kGy and 2.0 kGy, with either a low (12.0 and 5.6 ft/sec) or high (25.2 and 12.0 ft/sec) dose-rate.

All combinations of treatments for steaks and ground beef were analyzed immediately after irradiation (day 0). Samples were also stored at 7°C for 7 days in order to simulate consumer storage, followed by temperature abuse at 25°C for 2 days to simulate mishandling of these products after purchase. Preliminary studies have shown that storage of fresh meats at 2-4°C (simulate wholesale/retail storage) increased TPC by only approximately 1.0 log CFU/g, therefore 7°C was directly used in this study to simulate temperature of household refrigerator.

Physical and chemical analyses

Measurements of pH, lipid oxidation (TBA), and color were conducted on uninoculated samples except for those products where the total plate counts showed obvious spoilage ($>10^7$ CFU/g) or when obvious off-odor developed. Sample pH was measured using a slurry of 10-g of meat with 100-ml distilled water, a pH meter (Fisher Accumet Model 925, Fisher Scientific, Pittsburgh, PA) and a sealed combination electrode with silver/silver chloride reference (Omega Engineering, Inc., Stamford, CT). Measurement of thiobarbituric acid (TBA) values, an index of lipid oxidation, was

performed by the method of Tarladgis et al. (1960). Duplicate readings were recorded for each sample for pH and TBA tests. Color (L, a, and b) measurements were made with a HunterLab Labscan spectrophotometer (Hunter Associated Laboratories Inc., Reston, VA). An average value was obtained from three random locations on each sample.

Microbiological analyses

Separate sets of samples were used for microbiological analyses on sampling days. A 25-g sample was aseptically collected, and homogenized for 2 min in a sterile bag containing 225-ml of sterile 0.1% peptone water in a Stomacher lab blender (Model 400; Tekmer™ Co., Cincinnati, OH). Each sampling solution (1-ml) was added to tubes containing 10-ml enrichment broth to determine the presence of bacteria that might be too low to be enumerated by direct plating. Listeria enrichment broth (UVM, DIFCO), peptone sorbitol bile broth (PSBB) or modified EC broth (DIFCO) containing novobiocin (Sigma Chemical Co., St. Louis, MO) were used to enrich for *L. monocytogenes*, *Y. enterocolitica*, or *E. coli* O157:H7, respectively.

In most inoculated samples, cell counts were high (as expected) and could be enumerated by direct plating. Serial dilutions were prepared according to recommended microbiological procedures (Vanderzant and Splittstoesser, 1992), surface plated onto Plate Count Agar (PCA, DIFCO), and incubated aerobically at 25°C for 48 hr to enumerate total plate counts. *L. monocytogenes* was enumerated by plating onto Modified Oxford agar (MOX, DIFCO) containing Colistin sulfate-Moxalactam antimicrobic supplement (DIFCO), followed by incubation at 35°C for 48 hr. Typical *Listeria* colonies surrounded by a black zone were identified by Gram stain, catalase, oxidase, tumbling motility at 25°C, and confirmed by Micro-ID Listeria (Organon Teknika, Durham, NC). *Y. enterocolitica* was enumerated by plating on Yersinia

selective agar (DIFCO) containing Cefsulodin-Irgasan-Novobiocin antimicrobic supplement (CIN, DIFCO), and incubated at 25°C for 48 hr. Typical red "bullseye" colonies with transparent borders were identified by LAIA (LIA+α-arginine) slants and confirmed by Enterotube® II (Roche Diagnostic Systems, Montclair, NJ). *E. coli* O157:H7 can not utilize sorbitol thus will form white or translucent colonies on MacConkey sorbitol agar (MSA, DIFCO) after incubation at 35°C for 24 hr. Presumptive isolates were identified serologically with an agglutination test using O157 antisera (Oxoid, Unipath Co., Columbia, MD). Typical colonies on these plates were counted with a Darkfield Quebec colony counter (American Optical Co., New York, NY).

Sensory color and odor evaluation

Only uninoculated samples were used for color and odor evaluation which was conducted at day 0. A 10-member sensory panel evaluated samples by using a 5-point descriptive scale for surface color (1 = dark brown; 5 = dark red) and odor (1 = pleasant, no off-odor; 5 = extreme off-odor). Samples were temperature tempered at room temperature for 2 hr, unwrapped, and placed in ziploc pouches for evaluation. Ten steaks or five ground beef samples were evaluated at each session.

Statistical analyses

The experiment of steaks included 40 treatment combinations; two packaging treatments, four inoculation treatments, and five irradiation treatments (2 x 4 x 5 = 40). Measurements of pH, TBA, Hunter color L, a, b and sensory evaluation were only conducted on uninoculated samples; thus both included 10 (2 x 1 x 5) treatment combinations. Microbiological, physical and chemical analyses were conducted at day 0, 7, and 9; while sensory evaluation only conducted at day 0. Three replications were conducted. Total number of steaks used was 480 [(40 x 3 + 10 x 3 + 10 x 1) x 3].

The experiment of ground beef included 15 treatment combinations; four inoculation treatments, and five irradiation treatments ($4 \times 5 = 20$). The same sampling design as steaks, therefore the total number of 25-g ground beef used was 240.

Microbiological data were transformed into logarithms of the number of Colony Forming Unit/g ($\log_{10}$ CFU/g). Average data and standard errors were calculated from three replications. Each sampling time (day 0, 7, and 9) was analyzed separately due to the different temperatures used for storage. Physical, chemical, microbiological, and color/odor data were analyzed by using the Statistical Analysis System (SAS Institute Inc., 1986). The analysis of variance (ANOVA) procedure was used to detect the significances of replications, packaging (loins and steaks only), dose, and packaging by dose (loins and steaks only). Dose-rate and other combination effects were also evaluated. If no significant difference existed between low and high dose-rates, data were averaged for analyses. Comparisons of means was based on Duncan's multiple range test.

Results and Discussion

Effects of dose-rate on microorganisms and quality attributes

Some dose-rate effects on microorganisms were observed (data not shown). The differences, however, were all within 2 log and were not consistent, with both high and low dose-rates causing lower cell counts. On the other quality attributes, no significant dose-rate effect ($P>0.05$) was found between samples (data not shown). However, using a single radiation source means that dose-rate differences that can be obtained are relatively limited. There was more than a two fold difference between low and high dose-rates. The dose-rates may not have been significantly different to make a difference

in the numbers of survivors to irradiation. It is suggested that further studies using a wider range of dose-rates are needed to determine possible dose-rate effects.

Since no significant difference caused by two dose-rates used in this study, data were averaged and analyzed.

Effects of irradiation on microorganisms

Listeria monocytogenes Irradiation effectively reduced the number of *L. monocytogenes* in both beef steaks and ground beef. In steaks, there was only a 1 log cell reduction of *L. monocytogenes* by low dose (0.6 kGy) irradiation, compared with a 3 log cell reduction by medium dose (1.5 kGy) (Fig. 1). Storage at 7°C was effective in suppressing the growth of this organism in all samples, with no increasing counts observed during the storage period. Also, cell counts of irradiated steaks were kept lower than the controls throughout the storage period, especially for the medium-dose treated samples. After temperature abuse, growth rates of injured cells were faster than other treatments. Varabioff et al. (1992) showed that *L. monocytogenes* can survive well in chicken stored under vacuum or modified atmosphere (increased CO_2) after irradiated at 2.5 kGy compared with samples packaged in air. In our study, no significant difference was seen in the number of survivors of *L. monocytogenes* regardless of storage atmosphere. This may be because we used lower (0.60 or 1.5 kGy) doses, which allowed for more survivors to be present to begin with.

There was a significant difference in the number of survivors in ground beef according to dose level (Fig. 2). Low dose (0.80 kGy), and medium dose (2.0 kGy) irradiation reduced the number of *L. monocytogenes* by 1.5 log and 5 log, respectively. No obvious growth was observed during 7°C storage for all samples. Medium dose irradiation decreased the number of *L. monocytogenes* cells to a level less than 1 log CFU/g, and no survivors were detected after the temperature was elevated to 25°C.

Growth of this organism was totally masked by the high number (7.0 log CFU/g) of mesophilic organisms which existed in the spoiled ground beef. Comparing the effect of medium dose irradiation on survival of *L. monocytogenes* in steaks (1.5 kGy) vs. ground beef (2.0 kGy), we found that irradiation at 2.0 kGy was effective in reducing this pathogen in both products. However, surviving cells injured by low dose irradiation could proliferate during temperature abuse. Knabel et al. (1990) showed that storage under anaerobic conditions resulted in an increase in the number of *Listeria* spp. survivors to a heat treatment compared with storage under air. In our study, MOX plates were incubated aerobically, thus cell counts of this organism may have been lower than if the plates had been incubated anaerobically. However, there was no significant change in cell counts during 7 days of storage in both vacuum and air atmospheres. This can be explained by the fact that the storage period was too short, thus no recovery effect by atmosphere change was observed.

Yersinia enterocolitica   There was no significant difference in growth of *Y. enterocolitica* in steaks, regardless of packaging atmosphere (Fig. 3). This organism was eliminated to undetectable levels by 1.5 kGy irradiation and no further growth was observed during storage. Irradiation at 0.60 kGy only suppressed cell growth at the beginning of the storage period, with growth of injured cells being observed during extended storage. Hanna et al. (1976) reported that no *Y. enterocolitica*-like organisms were isolated until 21 days of storage at 1-3°C, and isolation of these organisms was more frequent after 28 days of storage under vacuum conditions than under nonvacuum conditions. We did not find any difference on cell recovery between samples packaged under vacuum vs. air, probably due to the short storage period, as mentioned above.

In ground beef, almost all *Y. enterocolitica* cells were eliminated by irradiation, regardless of the dose (Fig. 4). This was probably due to this organism's poor ability to compete, as well as its sensitivity to irradiation.

Although swine are considered a major natural reservoir for pathogenic *Y. enterocolitica*, contamination of beef with this organism is possible (Ibrahim and Mac Rae, 1991; Andersen et al., 1991). However, the occurrence of *Y. enterocolitica* is usually low, since this organism is less competitive than others found in animals. In this study, only two non-irradiated steaks were positive for *Y. enterocolitica* after 7 days of storage. No irradiated steak was found to be positive for *Y. enterocolitica* at day 0 due to sensitivity of this organism to radiation, even when doses as low as 0.6 kGy were used. These results confirm those of El-Zawahry and Rowley (1979), who reported that a dose of 1 kGy is adequate to eliminate *Y. enterocolitica* in raw meat.

***Escherichia coli* O157:H7**   *E. coli* O157:H7 was completely eliminated by irradiation at 1.5 kGy, with no survivors being detected on steaks during the storage period (Fig. 5). At least 1 log *E. coli* were reduced by 0.6 kGy irradiation, and the cells continued to grow during storage at 7°C and 25°C. Therefore, low dose irradiation can provide increased safety to products but only if they are not heavily contaminated with this organism. This organism is usually present in meat in low numbers, with less than 15 organisms per gram (USDA, 1993). After temperature abuse, growth of cells in the control set was suppressed to undetectable levels, regardless of storage atmosphere. It was possibly caused by accumulated CO_2 in packages, which was toxic to this organism. Spoilage occurred after 2 days at the abuse temperature, regardless of the packaging method, as evidenced by total plate count (TPC) of 10^7 cells/g (data not shown). No effect of air vs. vacuum packaging was found in this study, which is consistent with a study of Thayer and Boyd (1993). They also reported that growth of

Salmonella spp. (Thayer and Boyd, 1991) was not affected by air in the storage environment when irradiated in mechanically deboned chicken meat. They suggested that the mass of the meat probably masked any oxygen effect on the cells, if indeed present.

In ground beef, *E. coli* O157:H7 was also eliminated by medium dose (2.0 kGy) irradiation and not many cells were recovered during storage at 7° or 25°C (Fig. 6). There was only a 2 log cell reduction after irradiation at 0.8 kGy. Cell number was decreased during storage at 7°C. However, this organism proliferated rapidly after temperature abuse and reached the same number of survivors as controls.

More than half of the outbreaks of *E. coli* O157:H7 infections have been attributed to ground beef (Doyle, 1991). This organism is heat sensitive, therefore heating ground beef sufficiently to kill Salmonellae should also kill *E. coli* O157:H7. FDA recommends that hamburgers need to be cooked to a minimum internal temperature of 68.3°C (155°F) instead of 60°C (140°F), which is not sufficient to kill this organism (Mermelstein, 1993). Irradiation at 2.0 kGy can decrease the risk caused by *E. coli* O157:H7.

When medium doses were applied, *Y. enterocolitica* and *E. coli* O157:H7 were both reduced to undetectable levels, while some *L. monocytogenes* cells still survived (Fig. 1-6). These results confirm that *L. monocytogenes* is more resistant than the other two pathogens (Thayer, 1993).

Uninoculated samples   The effect of irradiation on uninoculated samples was also monitored. Except for very few steaks that were found to contain *Y. enterocolitica*, no *L. monocytogenes* or *E. coli* O157:H7 were found in these uninoculated samples. Enumeration of TPC is an index of total microbial load in meat, which may be used as routine checks for quality control purpose. From our results, TPC were reduced by 2-3 log immediately after irradiation at 1.5 kGy in steaks packaged

under vacuum or in air (Fig. 7). Samples irradiated at 0.60 kGy resulted in a reduction in the TPC of 1 log in vacuum packaged steaks, but in 2 log cells when packaged in air. This indicates that irradiation may be more effective in reducing TPC when oxygen is present, possibly due to an increase in the formation of oxygen-related free radicals which can affect microorganisms. Dose effects during 7°C storage was significant (P<0.05), with medium doses being more effective. However, after temperature abuse there was no difference (P>0.05) between any of the samples, regardless of dose. Thus, fresh beef irradiated at doses up to 1.5 kGy still requires adequate refrigeration to ensure safety and long shelf-life.

The initial TPC of ground beef was 7.0 log CFU/g (Fig. 8), which was higher than beef steaks (3.5 log CFU/g). Low (0.80 kGy) and medium (2.0 kGy) doses of irradiation caused 1.5 and 3.0 log reduction of TPC, respectively. When stored at 7°C, TPC of irradiated samples increased but remained less than 7.0 log CFU/g. However, non-irradiated samples had already spoiled (reached 8.5 log CFU/g) after 7 days of storage at the same temperature. After 2 days of storage at 25°C, samples irradiated at 0.80 kGy also spoiled, while samples irradiated at 2.0 kGy were at threshold (7.0 log CFU/g). This indicates that high contaminated meat samples, especially ground meat, may require a higher dose of irradiation to effectively reduce the contaminants and extend the shelf-life.

Ground beef usually spoils faster than beef loins or steaks due to more contamination in the former due to processing steps and a larger surface-to-mass ratio. Some competitive pathogens can survive in meat with high TPC. Refrigeration or frozen storage are important to suppress the high initial total count. However cooking temperature and time are probably even more critical because opportunity of contamination by pathogens is quite high.

Low temperature and vacuum packaging are of utmost importance to maintain high microbiological quality of meat. Longer shelf-life can be obtained when beef steaks are stored at lower temperature (4°C or lower) than 7°C. Lebepe et al. (1990) reported that 3.0 kGy radiation extended the microbiological shelf-life of vacuum-packaged pork loins stored at 2-4°C to more than 90 days. In our study, no significant difference (P>0.05) in TPC was observed between packaging environments when stored at 7°C. This may be because the storage time was relatively short (only 7 days).

Effects of irradiation on quality attributes

Some pH differences were observed in steaks (Table 1) and ground beef (Table 2), but may have been caused by normal variations in the meat.

Lipid oxidation causes major off-odor and deterioration of meat quality. In steaks, TBA values were increased after 7 days of storage on all samples, and air packaging showed more lipid oxidation than vacuum packaging. Vacuum packaging is superior to air for inhibiting lipid oxidation because less oxygen is available inside the pouch. TBA values were not positively correlated to microbial load. This was probably because microbial degradation of malonaldehyde and other thiobarbituric acid reactive substances (TBARS) occurred in the meat samples (Moerck and Ball, 1974). TBA values of ground beef were greater than 2.0 at day 0, and there was no significant difference (P>0.05) in these values between treatments (Table 2). Even though the TPC levels were significantly reduced, irradiation could not prevent or mask this lipid oxidation. After 7 or 9 days of storage, irradiated samples showed higher TBA values than those of controls. Irradiation may have delayed microbial degradation of malonaldehyde and other TBA reactive substance resulting in higher TBA values than the control. However, the validity of using TBA as an index for lipid oxidation of meat products, following irradiation and during storage, needs to be further investigated.

No significant (P>0.05) color difference of steaks was observed by the Hunter method (Table 1). Also, sensory evaluation showed no color difference (P>0.05) between control and irradiated samples (Table 3). Irradiated samples, regardless of packaging methods, produced a consistently higher off-odor score compared with controls, but the difference was not statistically significant (P>0.05). Vacuum-packaged beef has a limited shelf-life even in the absence of a high level of contaminating microorganisms (Egan and Shay, 1982) due to its "liver-like" off-odor development by predominantly lactic acid bacteria. After irradiation, lactic acid bacteria were predominant and may have enhanced this odor defect. However, this unattractive odor may have dissipated fast after opening of the package and could account for the inability of panelists to consistently identify it. Also olfactory adaptation or fatigue may have resulted in this deviation to some extent. For ground beef, although some values for color L, a ,b measurements were significantly different (P<0.05) (Table 2), no difference (P>0.05) in sensory color or odor were detected by panelists (Table 4).

Conclusions

Fresh beef, especially ground beef, is easily contaminated during processing. Irradiation, followed by refrigeration was found to be an effective way to reduce initial microbial loads, extending the shelf-life of meat without affecting its sensory quality. Also, irradiation increased meat safety by eliminating pathogens. Dose-rate was not a factor affecting survival of microorganisms in this study.

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Table 1. Effects of irradiation on selected characteristics of steaks packaged in air (A) and in vacuum (V) during storage at 7°C (day 0-7) and 25°C (day 7-9)

	Days of storage	Irradiation Dose (kGy)					
		0.0		0.60		1.5	
		A	V	A	V	A	V
pH	0	5.51^a	5.58^a	5.51^a	5.55^a	5.53^a	5.62^a
	7	5.51^a	5.48^a	5.57^a	5.45^a	5.53^a	5.51^a
	9	5.23^c	5.31^{bc}	5.35^{ab}	5.30^{bc}	5.43^a	5.42^a
TBA	0	0.65^a	0.27^{ab}	0.42^{ab}	0.14^b	0.58^{ab}	0.15^b
	7	2.33^a	0.76^b	2.43^a	1.23^b	2.58^a	1.25^b
	9	1.84^a	0.71^b	2.16^a	1.02^{ab}	2.07^a	1.03^{ab}
L value	0	30.0^a	28.8^a	29.3^a	29.3^a	29.6^a	27.2^a
	7	28.3^a	28.5^a	29.6^a	28.6^a	29.1^a	29.1^a
	9	30.8^a	31.3^a	29.7^a	29.9^a	29.5^a	29.0^a
a value	0	9.4^a	6.2^c	7.5^b	6.5^c	6.4^c	5.9^c
	7	7.3^a	7.1^a	7.1^a	7.0^a	6.7^a	6.6^a
	9	6.9^a	7.0^a	6.4^{ab}	6.8^a	6.0^b	6.5^{ab}
b value	0	5.8^a	4.6^b	4.8^b	4.0^b	4.8^b	4.1^b
	7	5.4^{ab}	4.4^c	4.8^{abc}	4.6^{bc}	5.5^a	4.8^{abc}
	9	4.6^a	4.7^a	5.1^a	4.7^a	4.7^a	4.5^a

[a-c]Values within each row with the same superscripts are not significantly different (p>0.05).

Table 2. Effects of irradiation on selected characteristics of ground beef during storage at 7°C (day 0-7) and 25°C (day 7-9)

	Days of storage	Irradiation Dose (kGy)		
		0.0	0.80	2.0
pH	0	5.53^a	5.48^a	5.52^a
	7	5.46^a	5.25^a	5.29^a
	9	6.51^a	5.63^b	5.52^d
TBA	0	2.23^a	2.08^a	2.08^a
	7	2.27^b	4.01^a	4.30^a
	9	2.22^b	5.28^a	6.19^a
L value	0	43.8^a	42.4^b	42.4^b
	7	44.3^a	45.4^a	44.3^b
	9	43.7^a	45.6^a	41.9^a
a value	0	8.1^a	7.7^{ab}	7.0^b
	7	6.9^a	6.5^a	6.4^a
	9	6.0^b	7.1^a	6.2^{ab}
b value	0	7.6^a	6.8^b	6.5^b
	7	7.1^a	7.4^a	6.8^a
	9	6.5^b	7.6^a	7.9^a

[ab]Values within each row with the same superscripts are not significantly different (p>0.05).

Table 3. Effects of irradiation on sensory color and odor characteristics of steaks packaged in air (A) and in vacuum (V)

	Irradiation Dose (kGy)					
	0.0		0.60		1.5	
	A	V	A	V	A	V
color	3.9[a]	4.1[a]	3.4[b]	3.9[a]	2.4[c]	3.6[ab]
odor	1.2[b]	1.5[b]	2.0[a]	2.2[a]	2.4[a]	2.2[a]

[a-c]Vaiues within each row with the same superscripts are not significantly different ($p>0.05$).

Table 4. Effects of irradiation on sensory color and
odor characteristics of ground beef

	Irradiation Dose (kGy)		
	0.0	0.80	2.0
color	3.2[a]	3.0[a]	2.8[a]
odor	3.1[a]	2.8[a]	3.0[a]

[a]Values within each row with the same superscripts are
not significantly different (p>0.05).
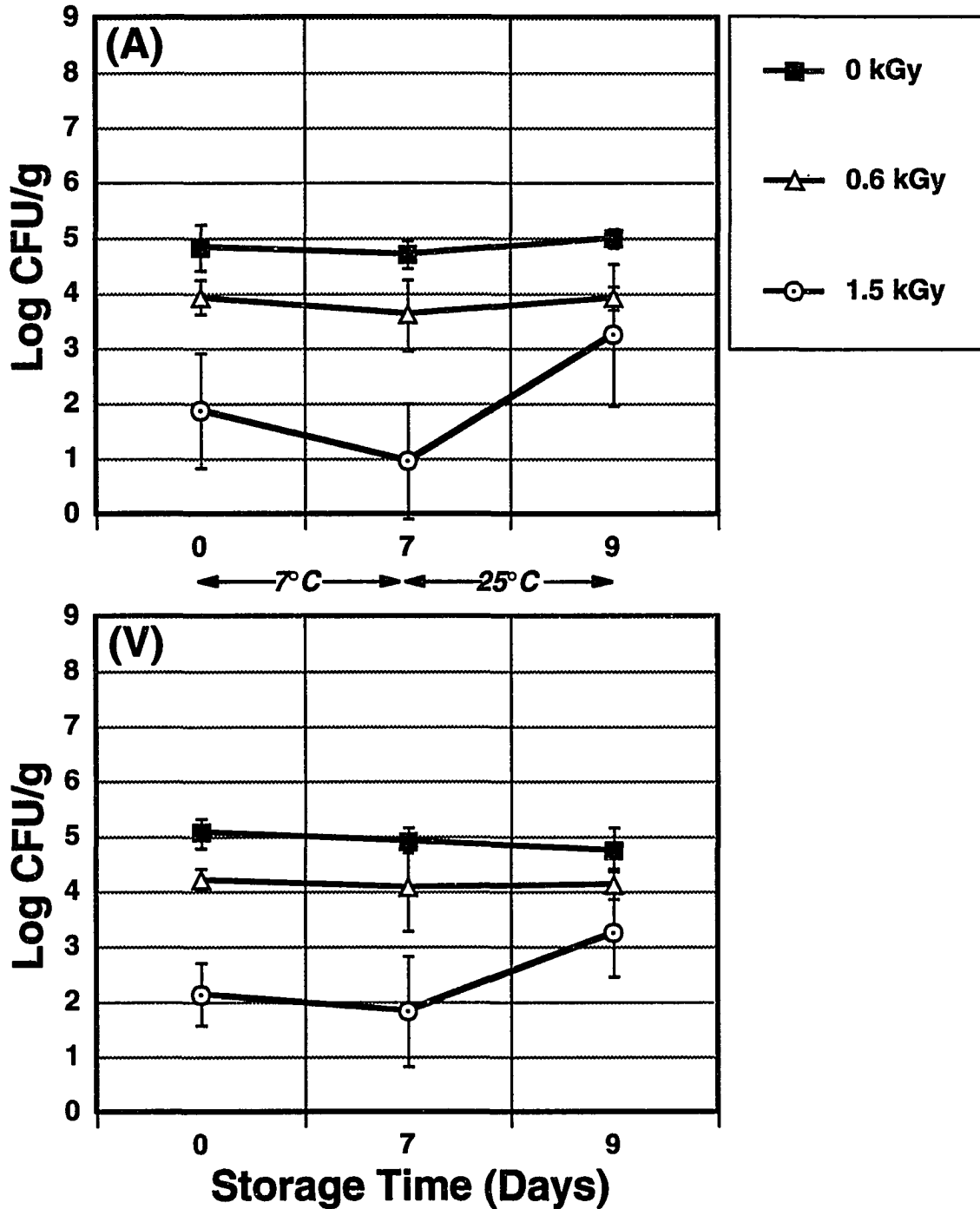
Figure 1. Growth of *L. monocytogenes* in air (A) and vacuum (V) packaged steaks treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
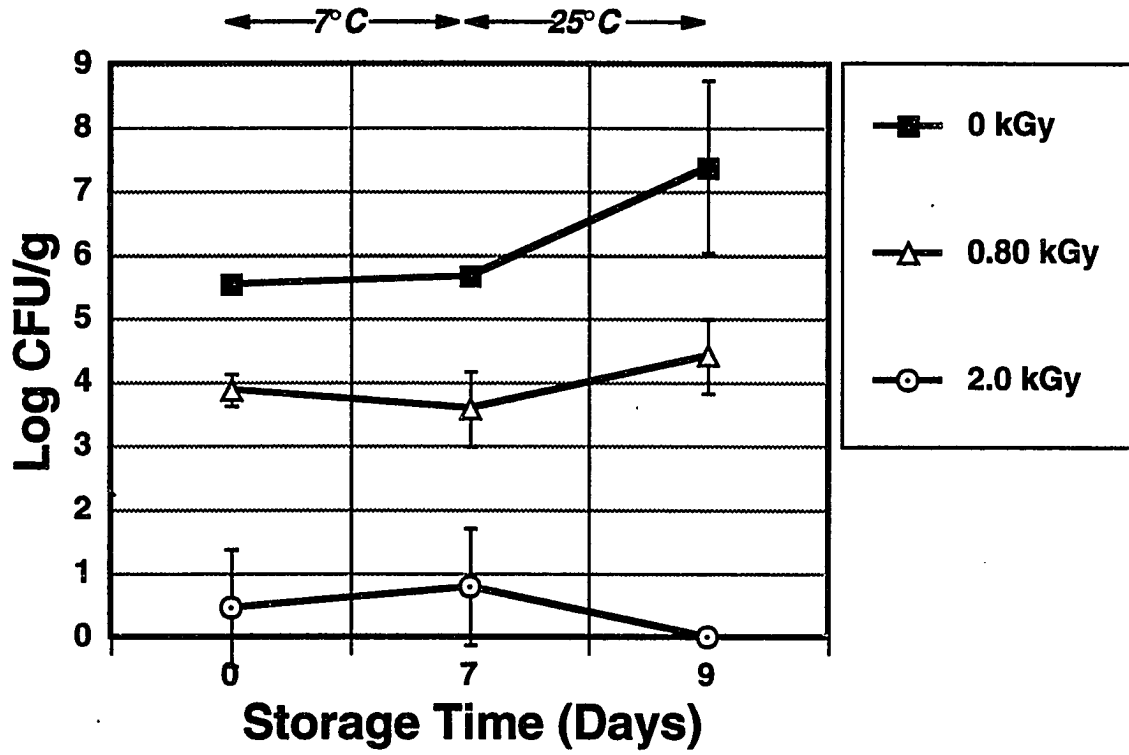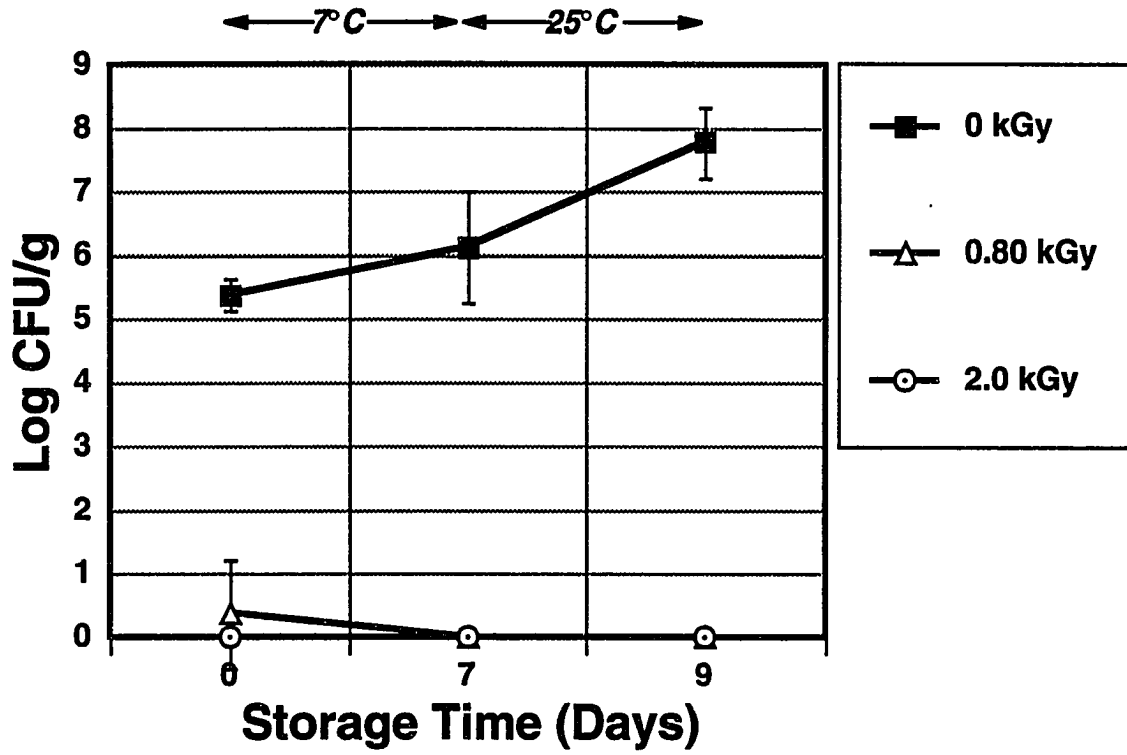
Figure 2. Growth of *L. monocytogenes* in ground beef treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
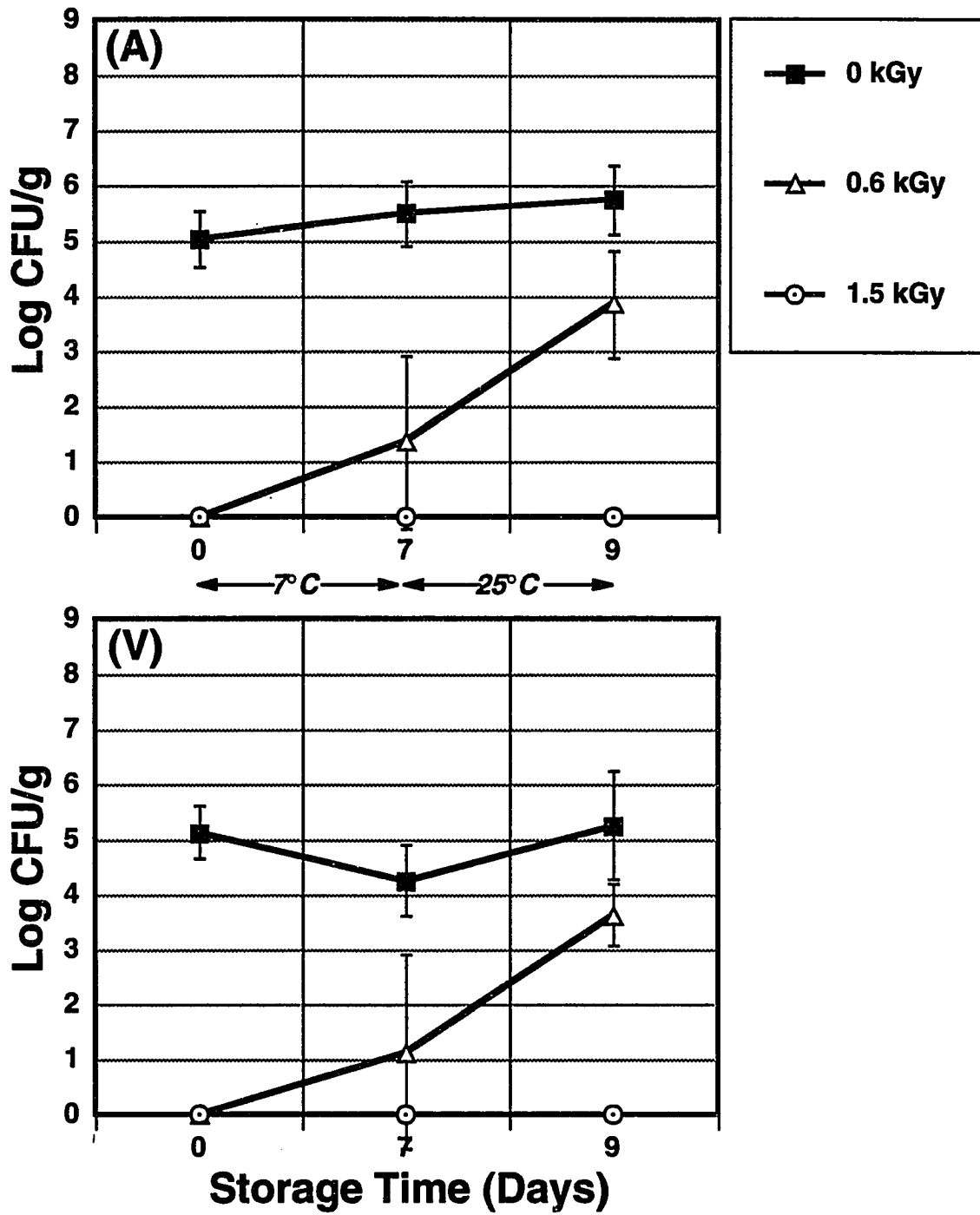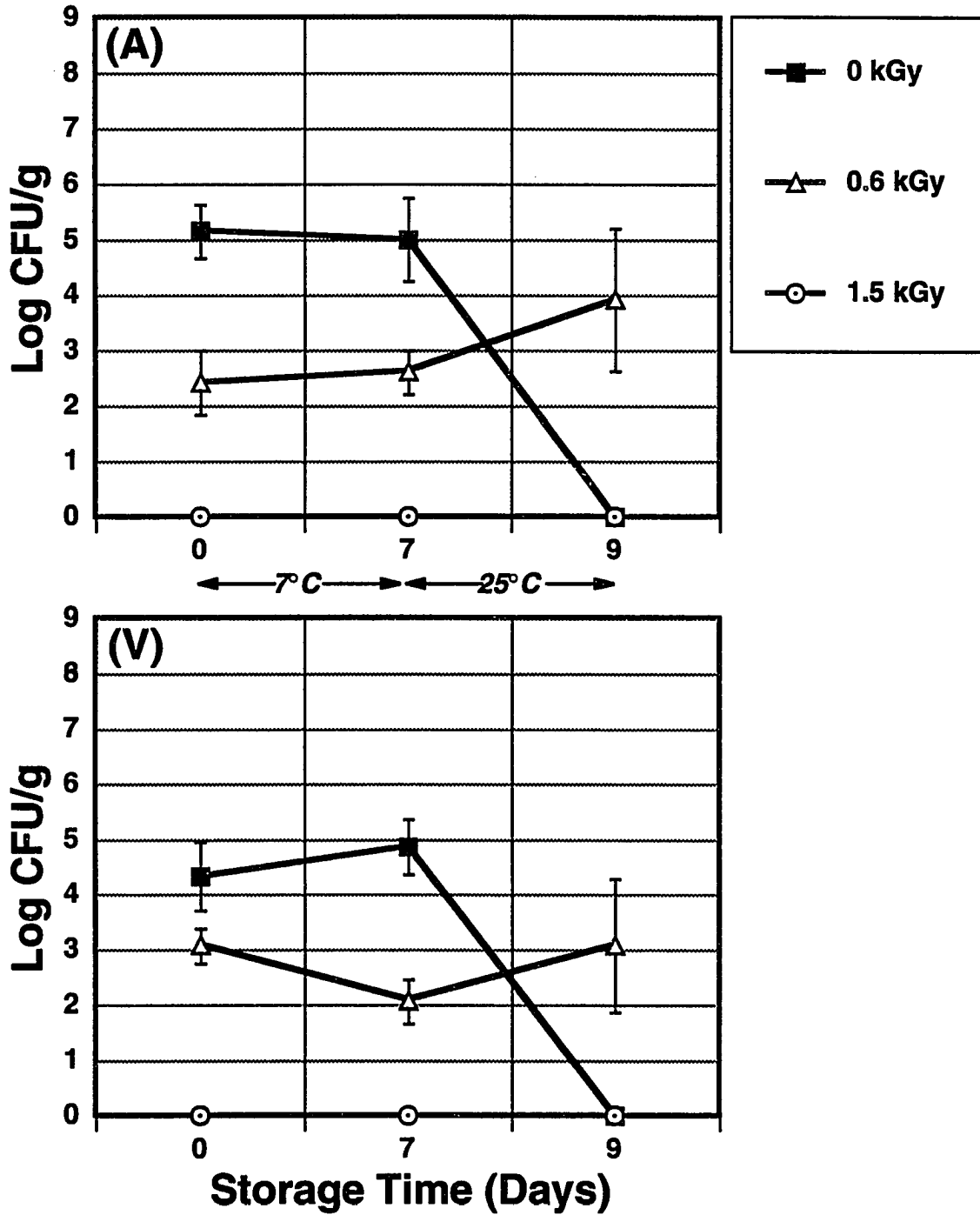
Figure 3. Growth of *Y. enterocolitica* in air (A) and vacuum (V) packaged steaks treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
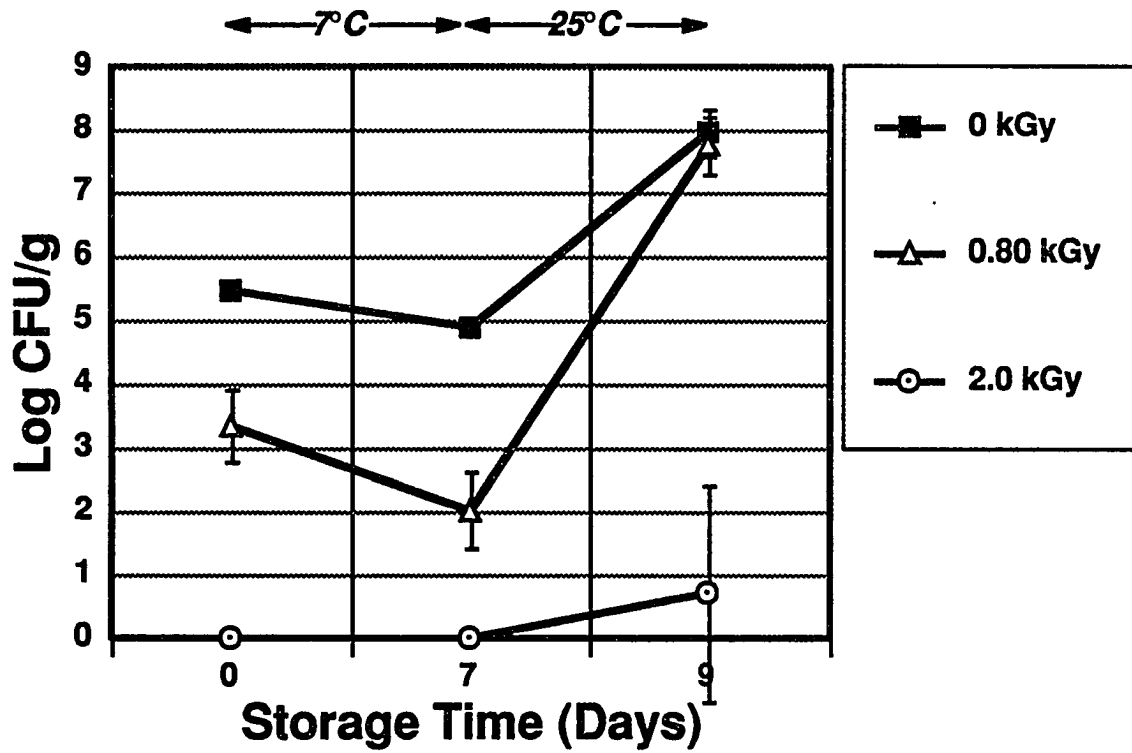
Figure 4. Growth of *Y. enterocolitica* in ground beef treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication

Figure 5. Growth of *E. coli* O157:H7 in air (A) and vacuum (V) packaged steaks
treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars
represent standard deviation of triplication

Figure 6. Growth of *E. coli* O157:H7 in ground beef treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
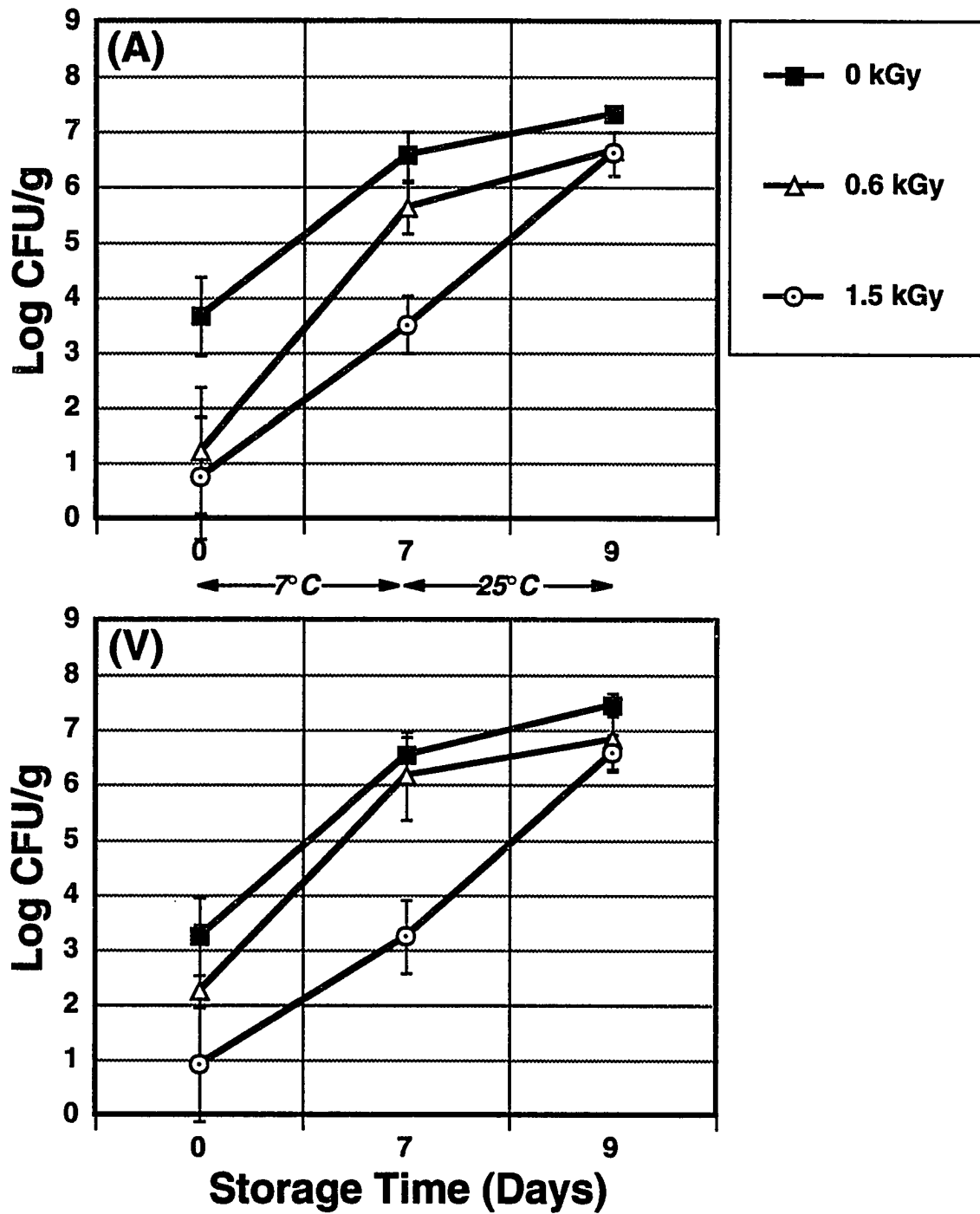
Figure 7. Total plate counts in air (A) and vacuum (V) packaged steaks treated with irradiation, stored at 7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of triplication
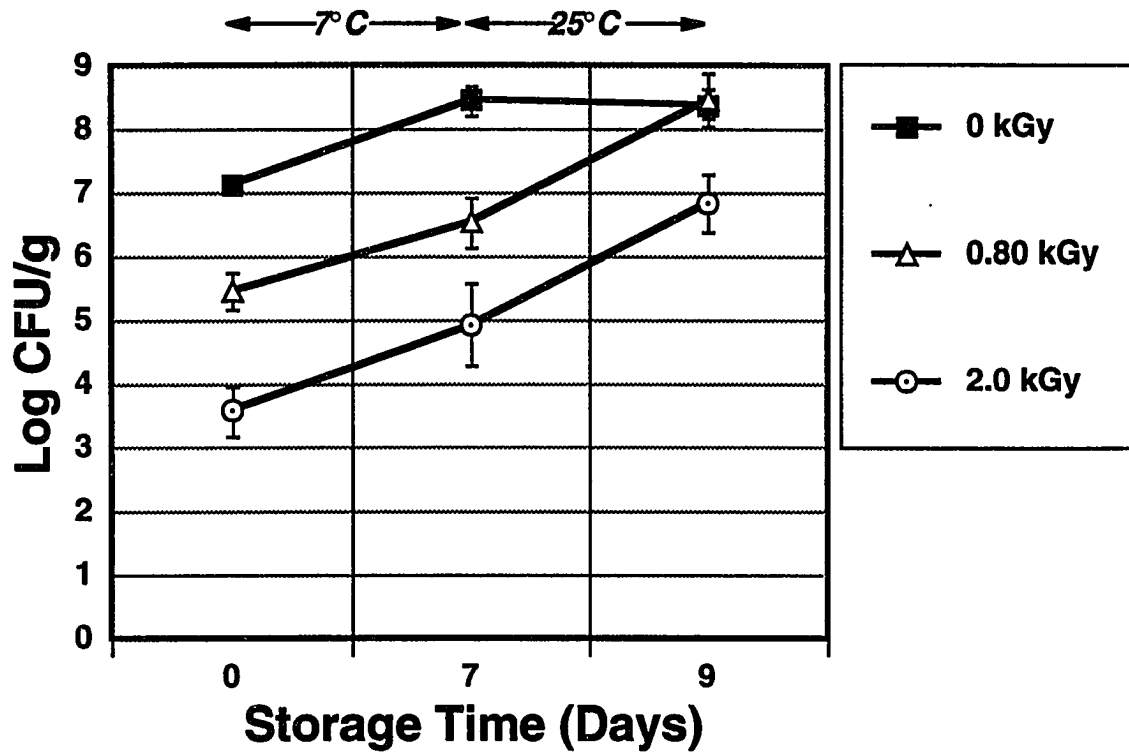
Figure 8. Total plate counts in ground beef treated with irradiation, stored at
7°C (day 0-7) and 25°C (day 7-9). Bars represent standard deviation of
triplication

CHAPTER V. GENERAL CONCLUSIONS

1. Medium dose irradiation was more effective in reducing total plate counts (TPC) and selected pathogens than low dose irradiation, with no significant changes in pH, TBA, and color attributes being detected.

2. Irradiation doses applied to eliminate *L. monocytogenes* and *S. typhimurium* were enough to also eliminate *Y. enterocolitica* and *E. coli* O157:H7.

3. Combination of brine pumping and low dose irradiation in chops was effective in reducing TPC after 7°C. Combination of salt/nitrite and low dose irradiation in hams was effective in reducing selected pathogens and TPC during 7°C storage.

4. Irradiation at appropriate levels was an effective means for decreasing the amount of salt and nitrite added in processed meat products.

5. Precooking was effective in reducing initial TPC in microwave-ready chops and hams.

6. Storage of meat and meat products at refrigeration temperature was effective in suppressing spoilage organisms and pathogens, even after irradiation.

7. No dose-rate effect was observed for the range of dose-rates used in this study.

8. Vacuum packaging did not affect the reduction or subsequent recovery of cells after irradiation.

Vacuum packaging, irradiation, refrigeration and other barriers offer a promising means to extend shelf-life and improve safety of meat and meat products using barrier concept technology.

It is suggested that conversion of electron-beam to X-ray mode, in which there is a loss of energy, could be used to achieve a very low dose-rate and achieve a larger

difference than was compared in this study. More research, with respect to microbiology, chemistry, and sensory quality, is required for red meats to demonstrate the advantages of irradiation and gain regulatory approval.

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