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# Irradiation and heating effects on microbiological, physicochemical, and sensory characteristics of ground pork

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Irradiation and heating effects on microbiological, physicochemical, and  
sensory characteristics of ground pork

by

Usana Navanugraha

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food Science and Human Nutrition  
Major: Food Science and Technology

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Iowa State University  
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DEDICATION

To my beloved parents, family, and friends.

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## GENERAL INTRODUCTION

From the early 1980's, the emergence of *Listeria monocytogenes* as a causative agent of human food-borne listeriosis has been recognized by the health authorities throughout the world. Nieman and Lorber (1980) described that, in adults, the disease is characterized by the onset of severe symptoms including meningitis, septicemia, primary bacteremia, endocarditis, non-meningitic central nervous system infection, and flu-like illness (fever, fatigue, malaise, nausea, cramps). Gastrointestinal symptoms are observed in approximately one-third of documented cases (Gellin and Broome, 1989). Humans shown to be at risk include pregnant women, neonates, organ-transplanted recipients, or those receiving immuno suppressive therapy. The sporadic incidence for listeriosis was found to be approximately 7 cases per million population per year, with an overall fatality rate of about 25% (Broome et al., 1990).

From 1979 to present, food-borne listeriosis has been linked to consumption of lettuce, carrots, radishes (Ho et al., 1986), coleslaw (Schlech et al., 1983), soft and semi-soft cheese (Farber et al., 1987), Mexican-style cheese (Linnan et al., 1988), Vacherin Mont D'or cheese (Bula et al., 1988), and milk (Fleming et al., 1985). Although it was suspected, *L. monocytogenes* has never been isolated as a causative agent for human listeriosis in meat and meat products. However, the Centers for Disease Control were able to link consumption of non-reheated hot dogs and undercooked chickens to sporadic cases of listerial outbreaks (Schwartz

et al., 1988). Because *L. monocytogenes* can grow at refrigeration temperatures, it could be a potential threat in ready-to-eat refrigerated foods. Moreover, *L. monocytogenes* is a non-fastidious organism and has been shown to survive a wide range of adverse growth conditions. As a result, the Food and Drug Administration (FDA), and the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA), established a zero tolerance for *L. monocytogenes* in 50 g (Farber, 1993) of ready-to-eat foods (Thayer and Boyd, 1995).

Pork has a relatively short storage-life as compared to other red meats (Boers and Dijkmann, 1994). Ground pork is subjected to additional handling steps that tend to increase the degree of contamination. Greater surface area and disruption of cell membranes make ground pork susceptible to oxidation as well as bacterial invasion. Spoilage of fresh meat is a complex phenomenon resulting from changes in muscle composition and changes in microorganisms. One early sign of spoilage of ground meat is the development of off-odors followed by tackiness indicating the presence of bacterial slime. Sliminess is due to both large masses of bacterial growth and the softening or loosening of meat structural proteins (Jay, 1992). At refrigeration temperatures, aerobically packaged fresh cut meat may have 5-7 days of storage life (Judge et al., 1989), while the shelf life of ground pork may be between 3-4 days.

Ionizing radiation is effective in destroying spoilage microorganisms and pathogens. On June 22, 1985, the FDA approved the irradiation of pork carcasses

and fresh pork cuts at radiation doses between 0.3 to 1.0 kGy to render pork safe from contamination of *Trichinella spiralis* (FDA, 1985). Doses required to destroy pathogenic bacteria are generally higher than those required to destroy parasites. These high doses of radiation can produce undesirable flavor changes in foods. Excessive thermal energy is required to preserve meats due to their low heat conductivity. The hurdle effect or barrier effect is increasingly important particularly for refrigerated foods. The concept is to use two or more mild processes which whenever applied individually are not adequate to destroy pathogenic bacteria and microorganisms that cause spoilage in foods. The ideal combination process would be one that works synergistically with each other to prolong the shelf life or add safety to foods while maintaining their quality.

Irradiation at higher temperatures has been shown to increase bacterial sensitivity (Grecz et al., 1971; Anellis et al., 1973; Thayer and Boyd, 1995). Heating with radiation appears to be a promising method to preserve many foods. By combining mild heat treatment with low dose radiation, the undesirable changes associated with either treatment may be minimized and the product quality improved. The objective of this research was two-fold. The first objective was to determine if heat and irradiation had a synergistic effect on reducing the number of survivors of *L. monocytogenes* Scott A and spoilage microorganisms. The second objective was to evaluate the physical, chemical, and sensory changes of ground pork receiving the combination treatment of heat and irradiation in air and vacuum packaging during storage at refrigeration temperatures.

## Dissertation Organization

This dissertation is an alternate style format consisting of a general introduction, a general review of literature, three papers prepared for publication, and a concluding summary. The three papers represent the work done by the first author to fulfill requirements for the degree of Doctor of Philosophy. The first two papers were prepared according to the Instructions for Authors as described in the Journal of Food Protection. The third paper was prepared for the submission to the Journal of Food Science and followed the "JFS Style Guide for Research Papers". These papers consist of an Abstract, an Introduction, Materials and Methods, Results and Discussion, a Conclusion, and References sections.

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## LITERATURE REVIEW

In the past decade, a number of psychrotrophic bacteria have emerged as food-borne pathogens resulting in several outbreaks. These outbreaks have resulted in the loss of human lives and increased the cost of health care. USDA estimated that the total cost of food-borne illnesses both parasitic and bacterial attributable to meat and poultry products is \$3,800-4,330 million annually (USDA, 1993). Several bacteria once considered as non-pathogenic to humans have emerged as human pathogens. The cause leading to the emergence of new food-borne diseases comes from the interaction of several factors. These factors are changes in pathogens, changes in behavior of consumers, and changes in food and the environment. Genetic changes in microorganisms can result in increased virulence. Human factors involve changes in eating habits, changes in food production and distribution systems, the ease of international travel, and an increase in the number of elderly and immuno-compromised individuals. In addition, new microbiological methods have been developed which are more sensitive in detecting very small numbers of pathogens.

### ***Listeria monocytogenes***

One of these new emerging pathogens is *Listeria monocytogenes*. Because of its ability to withstand adverse conditions, not common for other vegetative cells, it presents a dangerous health problem. The genus *Listeria* comprises five species *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. ivanovii*. Those

additional species not included in the generic description are *L. grayi*, *L. murrayi*, and *L. denitrificans* which are proposed to be reclassified (Stuart and Welshimer, 1974). The five species of listeria are characterized by the possession of antigens that give rise to 17 serovars. The somatic O antigens (1-4) give rise to 15 serovars, and there are 5 flagella or H antigens (a-e) (Jay, 1992).

Of the five listerial species, *L. monocytogenes* is the pathogen of most concern for humans. Although *L. ivanovii* can multiply in a mouse model, it multiplies less than *L. monocytogenes*, and up to  $10^6$  cells cause no infection in the mouse (Hof and Hefner, 1988). Other Listeria are nonpathogens, although *L. seeligeri* produces a hemolysin. The most significant virulence factor associated with *L. monocytogenes* is listeriolysin O (Jay, 1992).

*Listeria monocytogenes* is a small, Gram-positive, non-spore forming coccobacilli 0.4 to 0.5  $\mu\text{m}$  in size and tends to form short chains. In cultures incubated for 3 to 6 hours at 37°C, the bacillary forms predominate, but with more time the prevalent form is coccoid. In cultures of 3 to 5 days old, long filamentous structures that are 6 to 20  $\mu\text{m}$  or more in length often occur, especially in rough strains (strains of rough colony appearance and reduced virulence). At a lower incubation temperature (20 to 25°C), the motility of *L. monocytogenes* can be described as gently tumbling or slightly rotating motility by means of four flagella. At 37°C, only one flagellum is formed (Willett, 1984) and the motility is reduced.

*L. monocytogenes* is a non-fastidious microorganism that grows well in common media such as brain heart infusion, trypticase soy agar, and trypticase soy

broth. It grows best at 37°C under reduced oxygen and a 5 to 10 percent concentration of CO<sub>2</sub>. The mean minimum growth temperature on trypticase soy agar of 78 strains of *L. monocytogenes* was found to be 1.1 ± 0.3°C with a range of 0.5 to 3.0°C (Junttila et al., 1988). This bacterium is known to be relatively heat resistant for a vegetative cell. The maximum growth temperature for listeriae is approximately 45°C (Jay, 1992). *Listeria* spp. are catalase positive, oxidase negative, methyl red positive, Voges-Proskauer positive and indole negative (Lovett, 1990).

The growth of *Listeria* spp. is influenced by a combination of several factors. For example, the growth requirement of a bacterium is a function of temperature of incubation, nutrient composition of growth substrate, water activity ( $a_w$ ), presence or absence of oxygen, salts and/or inhibitors. *L. monocytogenes* can grow over the pH range of 4.5 to 9.8 (Chen and Shelef, 1992). At the same pH in tryptic soy broth, the antimicrobial activity of various acids are acetic acid > lactic acid > citric acid > malic acid > HCl (Hatfield, 1989). Several kinds of salts such as sodium chloride, sodium (or potassium) lactate, and sodium nitrite, are added frequently to meats and meat products. It was suggested that bacteriostatic effect on some of these salts is caused by the reduction of  $a_w$ . *L. monocytogenes* can grow at  $a_w$  as low as 0.92 (Ryser and Marth, 1991).

### **Incidence of *L. monocytogenes* in Nature**

*L. monocytogenes* is ubiquitous and can be found in soils, decaying vegetation, animal feces, sewage, silage, and water. Because of its resistance to adverse conditions, this organism can be found in natural surroundings throughout the world.

The first description of food-borne listeriosis in humans, with a direct link to animals, was provided in 1953 (Hird, 1987). In this instance, *L. monocytogenes* was isolated from a cow with listerial mastitis and from stillborn twins of a woman who had ingested raw milk from the same animal (McCarthy, 1990).

In the United Kingdom (UK), the number of incidents of listeriosis in sheep had increased from 53 in 1976 to more than 230 in 1983. In a 1971 survey, this organism was found in all 50 samples of sewage sludge, and river water. *L. monocytogenes* in sewage sludge was found to be in excess of 16,000 organisms/Kg (Watkins and Sleath, 1981). In Scotland, Fenlon(1985), was able to link the existence of *L. monocytogenes* in seagulls to their feeding locations. Gulls which lived at sewage disposal sites harbored 15% (99 total samples) of *L. monocytogenes* while only 4.5% of seagulls roosting elsewhere carry this organism.

In a sewage treatment plant in Iraq, *L. monocytogenes* was isolated at all stages of the treatment process including in the final discharge of a sludge cake (Al-Ghazali and AL-Azawi, 1986). Land, fertilized with this sludge, can spread the organism to animals being grazed on vegetation in the fields and finally to humans. The number of bacteria in the incoming raw sewage sludge ranged from 9 to 1,100

counts/g which were reduced to < 3 to 39 counts/g in the final discharge. This finding confirmed the fact that the organism could survive the adverse conditions in the stepwise processes of sewage treatment.

### **Prevalence of *L. monocytogenes* in Foods**

The widespread distribution of *L. monocytogenes* in nature and an association with domestic livestock makes the occasional presence of this bacterium in foods almost unavoidable. Foods from plant origin as well as those from animal origin are often found to be contaminated with *L. monocytogenes*. Since this organism can grow at refrigeration temperatures, it is a potential threat to humans whenever cross-contamination of foods occur. Undercooked and ready-to-eat foods are among the most hazardous of all as they may be consumed without subsequent heating

### **Dairy Products**

Listeriosis has been linked to consumption of soft cheese and semi-soft cheese (Farber et al., 1987; Linnan et al., 1988; Bula et al., 1988) and milk (Fleming et al., 1985). Previous investigations have identified sheep as a major reservoir of *Listeria* in nature (Donnelly, 1994). Cattle and goats are the domestic animals most frequently afflicted by listeriosis (Gray, 1960). *L. monocytogenes*, isolated from feces and milk of healthy and convalescent cattle, sheep, and goats (Hird and Genigeorgis, 1990), may enter into human food chain. Due to its wide range of growth temperature, pH and  $a_w$ , it may be expected to survive in raw milk and dairy products for a long time. For example, strain Scott A and V7, inoculated at the

levels of  $10^4$ - $10^5$  cells/g, survived in cottage cheese for 28 days at 3°C and 18 days during the camembert ripening process (Ryser et al, 1985; Ryser and Marth, 1987).

In the US, 4 to 12% of 121 to 350 samples of raw milk surveyed were contaminated with *L. monocytogenes* (Liewen and Plantz, 1988; Lovett et al., 1987; Hayes et al., 1986). It has been debated whether this organism can survive the pasteurization process after an incident of listeriosis linked to the consumption of pasteurized milk. Later studies indicate that it is unlikely that this organism can survive the pasteurization process. Jay (1992) stated that standard pasteurization protocols for milk were adequate for destroying *L. monocytogenes* at levels of  $10^5$  -  $10^6$ /ml, whether freely suspended in milk or in intracellular state. In the case of an outbreak linked to pasteurized milk (Fleming et al., 1985), *L. monocytogenes* was never isolated from the incriminated milk. It was isolated only from raw milk samples supplying the dairy plant (Farber et al., 1989).

The incidents of at least 4 listeriosis outbreaks in the U.S. and UK in the years 1983 to 1987, resulting from the consumption of soft and semi-soft cheese, prompted the survey of *L. monocytogenes* in cheese in several countries. In 1987, the FDA examined 181 samples of more than 10 varieties of cheese made from raw milk and recovered *L. monocytogenes* in one sample of cheddar cheese (Archer, 1988). A survey, conducted in Switzerland, found *L. monocytogenes* to be present in about 5% of 897 total samples of cheese (Breer and Schopfer, 1988). All of which were soft or semi-soft rather than hard types of cheese. In West Germany, 10.7% of 1490 total samples of cheese contained *L. monocytogenes* (Terplan,

1988). Again, soft and semi-soft cheese accounted for most of the positive samples. The incidence of this organism in soft cheeses was found to be 10% of 222 samples in Britain (Pini and Gilbert, 1988), to 14.5% of 69 samples of imported products from France (Beckers et al., 1987).

Ice cream was among the three kinds of foods (ice cream, salami, and vegetables) suspected to have caused 36 cases of listeriosis in Philadelphia during 1986-1987. Although *L. monocytogenes* was not recovered from the suspected foods, the organism was recovered from the patients (Schwartz et al., 1989). As *L. monocytogenes* is a potentially hazardous organism in dairy products, a survey of its occurrence is useful to predict the chance of contracting listeriosis. Ice cream from manufacturers across Canada (114 out of a total 147) were sampled. Of 530 samples of ice cream and ice cream novelties products obtained at the manufacturer level, 2 samples (0.4%) were positive for *L. monocytogenes* (Farber et al., 1989). The authors also stated that the incidence of *L. monocytogenes* contamination of ice cream varied from 0 to 5.5%, with very low level of counts (1 to 15 CFU/g) usually being present.

### **Meats and Meat Products**

Fresh meat when packed and stored in a hygienic condition contains low bacterial counts. Although red meat is occasionally contaminated at a low level with *L. monocytogenes*, listeriosis has never been linked to the consumption of meat. Cooking may be attributable to the non-existing case of human listeriosis in meat although the potential hazard of cross-contamination exists.

Level of contamination of potential pathogens in food is necessary to determine the severity of the process used. In 1987, the Microbiology Division of the USDA-FSIS launched a national monitoring program for *L. monocytogenes* in both raw and ready-to-eat meat and poultry products. The results between 1987-1988 showed that 41 of 658 samples (16%) of raw beef were positive for the organism (Carosella, 1990). Green (1990), also from the FSIS monitoring program, reported the updated incidence of *L. monocytogenes* in raw beef to be approximately 7% from 1727 samples.

Ground meat, a product that is subjected to more handling steps, would be expected to have a higher incidence of contamination. In Canada, Farber et al. (1989), found 95% of 19 samples of ground pork and 77% of 22 samples of minced beef to be contaminated with *L. monocytogenes*. In Alberta, Tiwari and Aldenrath (1990) reported 7 out of 11 samples of ground meat to contain *L. monocytogenes*. A study in Maryland showed that about half of frozen ground beef (41 total samples) and pork sausage (23 total samples) were contaminated with *L. monocytogenes* (McClain and Lee, 1988).

A survey found ready-to-eat meats from various countries, including those in North America, to be contaminated with *L. monocytogenes*. These meats are suitable growth media for the microorganism and are often consumed without further cooking. Ready-to-eat meats in Canada were found to contain *L. monocytogenes* in 33% of 18 samples (Farber et al., 1988). In the United States, a survey from retail stores in Wisconsin showed that 4.5% of 110 samples of roasted

meats were positive for this organism (Johnson et al., 1990). A study in the UK showed that 27% of 102 samples of precooked, refrigerated chicken, were positive for *L. monocytogenes* (Kerr et al., 1990).

*L. monocytogenes* is tolerant to curing salts in sausage formulations.

Approximately half (52% of 23 samples) of pork sausages in Maryland contained this organism (McClain and Lee, 1988). In Canada, Farber et al., (1988), reported that *L. monocytogenes* was found in 16% of 96 lots of raw, dry-cured sausage. Jay (1992) summarized the incidence of *L. monocytogenes* in processed meats from some European countries. The author quoted the incidence of the organism in dry raw sausage and fresh raw sausage in France to be 22% (from 37 samples) and 10% (from 120 samples) respectively. Mettwurst in Germany and Austria were contaminated at the levels of 59% (of 30 samples) and 23% (of 100 samples) respectively.

The level of *Listeria* spp. contamination was found to be approximately  $10^3$  CFU/g in raw meat products (Farber et al., 1989), 0.03 MPN/g<sup>1</sup> (most probable number/g) in fresh sausage, to approximately 0.30-0.74 MPN/g in ground veal, and 0.74-4.27 MPN/g in lamb (Buchanan et al., 1989). There was very little information on the count of *L. monocytogenes* in meat in these studies. Most studies were performed on all listerial species counts. Another reason for this lack of information

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<sup>1</sup> Number of microorganisms based on a statistical approach of probabilities

is because the recovery of *L. monocytogenes* in several studies were performed on enrichment media.

### **Vegetables and Other Foods**

In 1981, in the Maritime Provinces of Canada, coleslaw from infected cabbages was found to be the causative agent of listeriosis which led to the death of 2 adults and 16 infants. Listeriosis was found to exist on the farm where the cabbage was fertilized with raw sheep manure (Schlech et al., 1983).

Heisick et al. (1989) from Minnesota, found a high incidence of *L. monocytogenes* in some vegetables that have direct contact with soil. Potatoes and radishes were found to be positive for *L. monocytogenes* at the level of 26% (132 total samples) and 30% (132 total samples) respectively. Vegetables that have less contact with soil seemed to have a lower level of contamination. Leafy vegetables tend to be less contaminated than the underground edible parts.

From a survey in retail stores in Ontario of vegetables grown in North America, no *L. monocytogenes* was found in lettuce (50 samples), celery (30 samples), tomatoes (20 samples), and radishes (10 samples) (Fleming et al., 1985). However, *L. monocytogenes* was found in 4 of 60 prepackaged refrigerated salads (Sizmur and Walker, 1988) and in 9% of lettuce heads (Steinbruegge et al., 1988).

Poultry meat is also contaminated with *L. monocytogenes*. In the U. K., a pregnant woman had a stillbirth and flu-like symptoms after eating cooked-and-chilled chicken (Kerr et al., 1988). During the same year, a female cancer patient, in Texas, developed sepsis caused by eating turkey franks contaminated with *L.*

*monocytogenes* (Wenger et al., 1990). Between 20 to 30% (Green, 1990; McClain and Lee, 1988) up to about 50% (Farber et al., 1989) of chicken parts were found to be positive with this organism. In Taiwan, half of the 16 samples of chicken carcasses were positive for *L. monocytogenes* (Wong et al., 1990). The incidence is higher in the UK when raw chickens were found to be contaminated with *L. monocytogenes* in 57% of 35 samples (Pini and Gilbert, 1988). A study in Denmark also showed a similar incidence of *L. monocytogenes* in poultry neck skins with 47% (17 samples) being contaminated (Skovgaard and Morgen, 1988).

Although there were some reports of *L. monocytogenes* in the feces, body fluids, and oviducts of asymptomatic chicken (Leasor and Foegeding, 1989) there had been no documented *L. monocytogenes* food-borne illnesses due to the consumption of eggs or egg products.

#### **Serotype Distribution in Meat, and Meat Products**

Serotyping has been used with biotyping to resolve the virulence properties of *L. monocytogenes* with other characteristics (Benedict, 1990). The serology of five listerial species, as described by Seelinger and Donker-Voet, is based on 15 'O' (somatic) and 5 'H' (flagella) antigens (Lovett, 1990). Sixteen (Lovett, 1990) or seventeen (Jay, 1992) serovars are distinguishable with the five common *Listeria* spp. while *L. grayi* and *L. murrayi* are not included in the system. Based on these antigens, serotypes are classified as: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7 (McLauchlin, 1987), 4f, 4g, 5, and 6 (Pearson and Marth, 1990). Lovett (1990), on the other hand, described 6a (4f) and 6b (4g) instead of 4f, 4g, and 6

considering that 4f and 4g antigens were not always present. Among these, serovars 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7 had been classified under *L. monocytogenes* .

Of 722 human cases of listeriosis in the United States, 59% were caused by serotype 4b and 18% by serotype 1/2a (McLauchlin et al., 1986). Gellin and Broome (1989) identified that serotypes 4b, 1/2a, and 1/2b are responsible for 90% of human listeriosis in the U.S. In a similar survey of strains from Great Britain over the period of 1967-1984, 807 cultures belonged to the serotypes 1/2, 3, and 4 (McLauchlin et al., 1986).

In general, the three most prevalent serovars isolated from foods in decreasing order are 1/2a, 1/2b, and 4b, while from human listeriosis 4b, 1/2a, and 1/2b are the most prevalent (Cossart and Mengaud, 1989). Similarly, Johnson et al. (1990) summarized the serotyping of *L. monocytogenes* in meat and poultry in six countries and reported that the most common serovars were 1/2a and 1/2b.

### **Microbiology of Fresh, Refrigerated Meats**

With the exception of external surfaces (hair and skin) and gastrointestinal and respiratory tracts, tissues of living animals are essentially free of microorganisms (Judge et al., 1989). In the conversion of muscle to meat, microorganisms can contaminate meat surfaces at any step in the process, i.e., exsanguinating, slaughtering, cutting, processing, and improper packaging.

Living animals have leukocytes and antibodies that effectively control infectious agents. However, after slaughter and exsanguination, these mechanisms are lost, therefore, hygienic conditions of subsequent processing and storage are required. Upon prolonged storage (after 36 hours) at refrigeration temperature, spoilage of internal organs may occur by genera in the Enterobacteriaceae family and anaerobes like *Clostridium perfringens* (Ingram and Dainty, 1971). Important genera of bacteria found on meat surfaces during aerobic, refrigerated storage are: *Pseudomonas*, *Achromobacter*, *Micrococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Flavobacterium*, *Proteus*, *Alcaligenes*, *Acinetobacter*, and *Moraxella*. Among these bacteria, the genera that are found to be the primary cause of spoilage are *Pseudomonas* and *Acinetobacter-Moraxella* spp. (Jay, 1992). The non-fluorescent *Pseudomonas* produce hydrogen sulfides which are responsible for the off-odor and green discoloration of aerobically stored meat (Dainty et al., 1983). Gram-negative flora predominates aerobically stored meat with the exception of *Brochothrix thermosphacta*, a Gram-positive psychrotroph that was isolated from beef stored under oxygen permeable conditions (Ayres, 1960) and in nitrogen at 0°C (Weidemann, 1965).

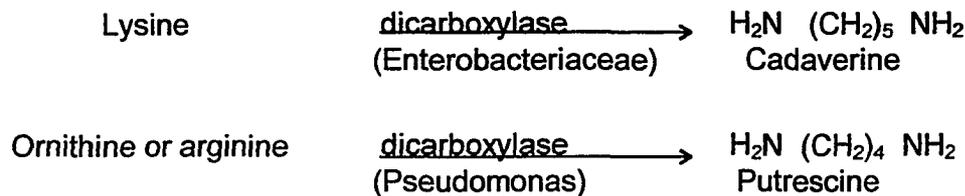
In vacuum packaging, some of the oxygen that remains is consumed by the aerobic microflora and by the fresh muscle tissue, whose continued respiration results in increased carbon dioxide in the package (Gardner et al., 1967; Seideman et al., 1979). Low-oxygen concentration (<5%) favors the growth of facultative

anaerobic lactic acid bacteria over that of aerobic bacteria; hence a shift toward a Gram-positive microflora (Grau, 1983; Lee et al., 1985).

When red meats are stored at 0-5°C under packaging conditions that restrict the entry of O<sub>2</sub> or if there is accumulation of CO<sub>2</sub>, the ultimate flora is dominated by lactic acid bacteria (Jay, 1992). In a study of vacuum-packaged beef and lamb stored for 28 to 35 days, the initial flora of *Corynebacterium* spp. and *B. thermosphacta* became dominated by lactobacilli (Hanna et al., 1977). High concentrations of CO<sub>2</sub> in meat packs also shift the flora from a heterogeneous one consisting of Gram-negative bacteria to one consisting primarily of lactobacilli and other lactic acid bacteria. Blickstad et al. (1981) found that over 90% of the flora in pork consisted of *Pseudomonas* spp. in aerobic storage. However, after 5 atm CO<sub>2</sub> storage, lactobacilli dominated. *Pseudomonas* spp. are more sensitive to CO<sub>2</sub> than *B. thermosphacta* which in turn are more sensitive than lactic acid bacteria (Erichsen and Molin, 1981). An increased concentration of CO<sub>2</sub> in vacuum packaging has little effect on lactic acid bacteria. Several authors identified lactobacilli as the predominant bacteria found on vacuum packaged meat (Ingram, 1962; Baran et al., 1969; Pierson et al., 1970). Jaye et al. (1962) suggested that lactic acid bacteria become dominant after 8 days of storage in vacuum packaged ground beef. Ordal (1962) also demonstrated that lactic acid bacteria, which represent a low proportion of the bacteria in initial counts, increased to over 50% of the population after 6 to 12 days of vacuum storage. Baltzer (1969) stated that vacuum packaging changed the bacterial flora by giving the lactic acid bacteria a

chance to outcompete against the pseudomonads. The author also noted that vacuum packaged meat had a slower increase in microbial spoilage counts, and lower final counts than did aerobic packaged meat. Due to the shift of microflora toward lactic acid bacteria, spoilage of vacuum packaged meat results in souring of the meat instead of putrefaction and slime formation.

Spoilage of meats at low temperatures is accompanied by the production of off-odor compounds such as ammonia, hydrogen sulfide, indole and amines. The production of these amines occurs as follows:



Cadaverine increased more than putrescine in vacuum-packaged meat while the reverse is true for aerobically stored samples (Edwards et al., 1985).

### **Effect of Ionizing Radiation on Microorganisms**

The most important purpose of applying ionizing radiation to meat is to destroy the pathogenic microorganisms while maintaining the “freshness” of foods. Ionizing radiation has been referred to as a “cold” process which implies that temperature increase is small and the product retains its fresh, uncooked appearance. Other benefits include the control of microbial spoilage, thus prolonging the shelf-life of meats. Ionizing radiation can effectively destroy pathogens. However, sterilization doses may adversely affect the eating quality of

foods particularly flavor and aroma qualities. Optimal radiation conditions (dose, packaging, irradiation temperature, treatment following radiation, etc.) can be chosen to serve the purpose of radiation application to a particular food item.

An international group of food scientists suggested the following terminology to describe different levels of radiation treatment of food (Goresline et al., 1964).

These terms: radappertization, radicidation and radurization, were defined (Anon., 1982) as follows:

Radappertization is:

‘Treatment of food with a dose of ionizing radiation sufficient to reduce the number and/or activity of viable microorganisms to such a level that very few, if any, are detectable by any recognized bacteriological or mycological testing method applied to the treated food. The treatment must be such that no spoilage or toxicity of microbial origin is detectable no matter how long or under what conditions the food is stored after treatment, provided it is not recontaminated.’

Radappertization is equivalent to “commercial sterility” as it is understood in the canning industry. Typical levels of irradiation are 30 to 40 kGy although dose ranges of 10 to 50 kGy are sometimes employed. Radappertization renders long-term shelf stability of products without refrigeration.

Radicidation is:

“Treatment of food with a dose of ionizing radiation sufficient to reduce the number of viable specific non-sporeforming pathogenic bacteria to such a

level that none is detectable in the treated food when it is examined by any recognized bacteriological testing method.”

Radicidation is equivalent to pasteurization and requires 2.5 to 10 kGy of radiation.

The product shelf-life is limited and refrigeration is required.

Radurization is:

“Treatment of food with a dose of ionizing radiation sufficient to enhance its keeping quality by causing a substantial reduction in the numbers of viable specific spoilage microorganisms.”

Radurization refers to a radiation process that substantially reduces spoilage microorganisms, though refrigeration is needed. Common dose levels are 0.75 to 2.5 kGy for fresh meats, poultry, seafood, fruits, vegetables, and cereal grains.

Ionizing radiation injures or kills microorganisms by both direct and indirect action. As a consequence of the radiation-induced changes, an injury and cell death may occur. Cell death is associated with the inability of cells to reproduce while cell injury may result in the recovery of microorganisms in an optimum growth conditions. The important target of radiation is the cell DNA. The size of the DNA molecule and its complexity make it especially vulnerable to change by radiation (Urbain, 1986).

In general, the radiation resistance of microorganisms in food varies inversely with the complexity of their morphology. The resistance of microorganisms to irradiation in food progressively increases in the following order: molds and yeasts <vegetative bacteria <sporulating bacteria <viruses. The Gram-

negative rods are among the most radiosensitive of all bacteria, and they are the principal spoilage organisms in foods (Jay, 1992). D-values<sup>2</sup> (decimal reduction dose) of *Pseudomonas* spp. were found to be in the order of 0.08 kGy (Patterson, 1988) and 0.13 kGy (Tsuji, 1983.). The Gram-negative coccobacillary rods belonging to the genera *Moraxella* and *Acinetobacter* have been found to possess degrees of radiation resistance higher than for all other Gram-negatives (Tiwari and Maxcy, 1972). Following gamma radiation doses of up to 2.72 kGy, the authors found that 73 to 75% of the surviving flora consisted of these related genera while only 8% was found in non-irradiated ground beef. Welch and Maxcy (1975) reported that the *Moraxella* spp. appear to be more resistance than *Acinetobacter* spp. with D-values in the order of 0.85 kGy (Patterson, 1988) and 0.25 kGy (Tsuji, 1983) respectively.

Low doses of irradiation shifts the microflora in stored meat toward Gram-positive microflora as well as in vacuum-packed chilled meat. Gram-negative bacteria are more sensitive to radiation than the Gram-positive bacteria. As a result, while *Pseudomonas* prevails in non-irradiated, refrigerated, aerobically stored meat, Gram-positive bacteria like lactobacilli and micrococci prevails in irradiated meat. *B. thermosphacta*, another contaminant often found in meat, is also more resistant to radiation than aerobic spoilage species such as *Pseudomonas*

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<sup>2</sup> Irradiation dose required to reduce the number of survivors by 1 log cycle

(Gardner, 1981). Occasionally, *B. thermosphacta* was found to dominate in low dose irradiation of beef (Wolin et al., 1957). In a study of low-dose irradiation on the microflora of vacuum-packed ground pork held at 5°C, Ehioba et al. (1988) found Gram-positive bacteria to dominate in irradiated pork whereas Gram-negative bacteria predominated the non-irradiated samples.

When irradiation was introduced, the 12-D (the process required to reduce number of viable cells or spores by 12 log cycles) concept of thermal processing was proposed to evaluate the radappertization doses required to sterilize low-acid foods (Schmidt, 1961). The 12-D concept is based on the classical studies by Esty and Meyer (1922) on the heat resistance of spores of *C. botulinum* when the bacteria were formerly classified in the genus *Bacillus*. The 12-D process for *C. botulinum* spores in pork loins was 43.7 kGy at -30°C (Grecz et al., 1965). This dose level is higher than the threshold dose of several kinds of meat. Bacterial toxins are more resistant to radiation than spores. The D-values for type A botulinum toxin exceeded 40 kGy in an anoxic and highly proteinaceous medium, but was only 1 kGy in phosphate buffer at pH 7.5 (Wagenaar and Dack, 1960).

Food irradiation research in the early days was concentrated in the radappertization dose range while low doses of irradiation in combination with other treatments has been investigated within the past 15 years. There is an increasing awareness of psychrotrophic pathogens in low acid, refrigerated food products in the U.S. market. *L. monocytogenes*, *Yersinia enterocolitica*, *Aeromonas hydrophila*,

*Escherichia coli* O157:H7, *C. botulinum* type E are examples of food poisoning microorganisms that can grow in refrigerated foods at temperature as low as 0°C. Among these, *L. monocytogenes* is probably the most radiation resistant species and *Y. enterocolitica* is the most sensitive species.

D-values depend on several factors. Most studies reported bacteria to be more resistant in the early stationary phase. Dion et al. (1994) found no significant difference in radiation resistance of *L. monocytogenes* 1A1, *S. aureus* ATCC 13565, *E. coli* O157:H7, *Y. enterocolitica* and *Vibrio parahaemolyticus* at different age. Other strains; namely, *L. monocytogenes* ATCC 19111 and *S. aureus* GD13 were significantly more resistant in the stationary phase (D-values-0.23 and 0.12 kGy) than in the exponential phase (D-values = 0.17 and 0.09 kGy respectively).

Complex food constituents were found to have a protective effect on bacterial cells toward irradiation. D-values of *L. monocytogenes* were higher in poultry minced meat (0.42-0.55 kGy) as compared to those in phosphate-buffered saline solution (0.32-0.49 kGy) (Patterson, 1989). Dion et al. (1994) reported higher resistance in chicken meat as compared to saline solution in *L. monocytogenes*, *V. parahaemolyticus*, *E. coli* O157:H7 (ATCC 35150), *S. typhimurium*, and *Y. enterocolitica*.

Higher irradiation dose range results in higher resistance found in *L. monocytogenes*. Seven mixed strains of *L. monocytogenes* in BNT medium (mixture of 0.4% nutrient broth (Difco) and 1.5% trypticase soy broth (BBL) with glucose) had an average D-value of 0.27 kGy when radiation resistance study was

performed in the dose interval of 0.00-0.05 kGy (Huhtanen et al., 1989). At a higher dose range of 0.00-0.20 kGy, an average D-value of 0.33 kGy was found. When mechanically deboned chicken meat was used, the higher resistance was also demonstrated at a higher dose range interval, e.g. D-value=0.27 kGy (dose range 0.00-0.50 kGy), D-value=0.77 kGy (dose range 1.00-2.00 kGy).

Bacteria show an increased radiation resistance when irradiation temperature is decreased, particularly below sub-freezing temperature. For example, D-values of *L. monocytogenes* were evaluated in mozzarella cheese and ice cream to be 1.4 kGy and 2.0 kGy respectively by gamma irradiation at -78°C (Hashisaka et al., 1989).

Other factors such as  $a_w$ , pH, oxygen, media and temperature of recovery all have influence on the radiation resistance of microorganisms. Following the stress of irradiation, optimum conditions are needed to recover injured cells, whose condition for recovery varies from one strain to another.

### **Effect of Ionizing Radiation on Quality of Meats**

Meats are spoiled by biochemical pathways that lead to rancidity and by microbial action. Irradiation contributes to food preservation by controlling the spoilage microorganisms and pathogens. Other meat constituents responsible for physical and chemical changes in meat, such as proteolytic enzymes, are resistant to radiation and need to be controlled by other preservation methods. Irradiation, particularly at a radappertization dose, may cause some undesirable changes in

meat. The most objectionable change is the "irradiated" flavor and odor in meats.

### **Flavor Odor and Aroma**

The exact nature of "irradiated" flavors has not been fully characterized and still remained a controversial issue (Nawar, 1983). Few, if any, objectionable odors or flavors are produced when fresh or cooked meat is irradiated at a radurization dose. At higher irradiation dosages (> 10 kGy), the irradiation odor/flavor may be described as 'scorched' or 'burnt', or as "wet dog" (Urbain, 1986).

As early as 1954, researchers had shown sulfur (S)-containing compounds in the water-soluble fractions of meats to be responsible for off-odor (Schweigert et al., 1954). Water extracts of beef irradiated to  $0.5-1.6 \times 10^6$  rep<sup>3</sup>, roentgen equivalent physical, (4.65-14.9 kGy) exhibited radiation-induced off-odor while none was found in the ether-extract and salt-extract fractions. The insoluble residue from the water extract showed a weak irradiation odor. The authors pointed out that methyl mercaptan and sulfur dioxide contribute to some of the off-odor and that they are formed from S-containing compounds of glutathione and cysteine. Beef tallow was also irradiated at a similar dose at refrigeration temperature, in air and its peroxide values were higher than in the non-irradiated controls but no irradiation off-odor was detected. Results from these experiments indicated that the precursor of the undesirable odoriferous compounds were water soluble and probably contained

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<sup>3</sup> An obsolete unit of absorbed dose of ionizing radiation equivalent to 93 erg/g

nitrogen and/or sulfur (Schweigert et al., 1954). They also indicated that the mechanism of irradiation flavor development was different from lipid oxidation. Following this work, S-containing compound of soluble protein and glutathione had been suspected as the main source of irradiated odor. Hedin et al. (1960) found off-odor resulting from the water soluble fraction from at least two separable proteins. The authors concluded that off-odor is associated with sulfur or its related compounds.

Merritt (1966) suggested the radiation impact on the protein and lipid molecules to form volatile compounds that are responsible for the odor/flavor changes in irradiated meat. A mechanism was proposed for a "direct cleavage" at all positions on lipid molecules followed by secondary collisions extracting secondary electrons. In the lipid fraction of beef irradiated to 60 kGy, homologous series of n-alkane (60%), n-alkene (40%), with traces of alkyne and alkadiene were formed. In non-irradiated control, almost no hydrocarbon compounds and only ethyl alcohol was present. In the protein fraction of irradiated meat, hydrogen sulfide, sulfur dioxide, carbonyl, acetone and methyl ethyl ketone were recovered in moderate amounts.

Nawar and Balboni (1970) irradiated 30% fat containing pork to 60 kGy and found hydrocarbon compounds to increase linearly with dose. The formation of hydrocarbons was unaffected by the fat nor by oxygen during irradiation. Again, lipid fraction contained a large amount of hydrocarbon, an odorless component, with the protein fraction containing primarily carbonyl compound. The meat fraction contained both hydrocarbons and S-containing compounds, alcohol, ketone, carbonyl compounds, and showed irradiated odor. Similar results had been

reported previously by Merritt et al. (1966) which showed irradiation odor in the lipoprotein fraction of irradiated (60 kGy) haddock.

Other authors recorded the decrease of glutathione by irradiation (Batzer and Doty, 1955; Ingram et al., 1959) and hydrogen sulfide formed (Ingram et al., 1959; Marbach and Doty, 1956). Martin et al. (1962) confirmed the hydrogen sulfide formation from irradiated glutathione by adding radioactive S-35 glutathione to ground beef. At 100 kGy, they found that 20% of hydrogen sulfide and 91% of methyl mercaptan had come from glutathione.

Oxygen-influenced carbonyl compounds (Scribney et al., 1955) had been proposed for radiation-induced off-odor. However, some increase in carbonyl levels is observed even when irradiation is carried out in nitrogen atmosphere in sealed containers.

Irradiation initiates free radicals and its mechanism to produce off-odors was once thought to be the same as lipid autoxidation. However, irradiated (60 kGy) and oxidized (1 week in copper catalyst) butter fat showed distinctly different kinds of odor, i.e. irradiated fat as opposed to oxidized odor. Irradiated butter fat contained a large amount of hydrocarbons but no carbonyl compounds even in air (Merritt, 1966). The oxidized fat contained high amounts of carbonyl compounds and very small amounts of hydrocarbons. This experiment suggests different mechanisms of irradiation and autoxidation on odor in meat. In addition, the formation of hydrocarbons is independent of oxygen content although the formation of oxidized components may be increased in the presence of air (Merritt, 1966).

The effect of oxygen to enhance off-odor induced by radiation has often been observed during storage in air but not immediately after irradiation. Proctor et al. (1954) found no significant increase in peroxide values in irradiated fat of beef, chicken, and hamburger immediately after 2 Mrep ( $10^6$  rep) either in air or under vacuum. Astrack et al. (1952) reported peroxide values in irradiated (1.5 Mrep) mackerel oil to be higher than in non-irradiated control during storage in air-sealed containers with limited headspace at 25°C. Peroxide values of samples packaged in nitrogen and under vacuum remained practically unchanged until day 50 of storage.

Effect of oxygen on oxidation of irradiated products appears to show after a prolonged storage period rather than immediately after irradiation. Diehl (1983) suggested that after-effect of irradiation depends on dose, atmospheric oxygen, food composition, temperature during irradiation, and storage temperature. With extended storage and/or cooking, the irradiation off-odor tends to disappear but the reverse is true for lipid autoxidation.

### **Color**

Purified myoglobin or water extracts of meats turn from red color of oxymyoglobin to the brown color of metmyoglobin after irradiation (Scribney and Schweigert, unpublished data, as cited from Schweigert et al., 1954). When fresh ground beef was irradiated to 145,000 rep in oxygen-impermeable casing, less discoloration (slightly faded red color) was observed. In air-packaged casing, however, the brown color of irradiated beef was found (Schweigert et al., 1954).

Irradiation with doses > 1.5 kGy in air causes a brown discoloration of meat much like the color of metmyoglobin. In the absence of air and at higher doses, a bright red color similar to color of oxymyoglobin is observed (Huber et al., 1953). In cooked meat, the greyish brown color of denatured metmyoglobin is changed to bright red (Tappel, 1957). The color of nitrate-cured samples changed to a desirable bright red after irradiation (Kamarei, 1981). The spectral characteristics of the brown pigment were similar to metmyoglobin and those of the bright red pigment were similar to oxymyoglobin with small differences in the position of the absorption maxima (Diehl, 1983).

Color of beef cuts irradiated to 5 kGy changed to metmyoglobin after 21 days of storage in vacuum and 21 days in vacuum plus 1 day in air (Urbain, 1973). Meat dipped in tripolyphosphate (10% w/w) for 30 sec retarded oxidation of oxymyoglobin to metmyoglobin both in irradiated and non-irradiated samples. Samples irradiated in excess of 3 kGy had higher metmyoglobin content than the non-irradiated control.

### **Texture and Water-Holding Capacity**

The overall impression of tenderness to the palate includes texture of the meat and involves three aspects (Bratzler, 1971). Firstly, the initial ease of penetration of the meat by the teeth; secondly, the ease with which the meat breaks into fragments; and thirdly; the amounts of residue remaining after chewing.

Lawrie et al. (1961) reported immediate effects of 50 kGy irradiation on beef and pork *longissimus dorsi* muscle to be an increase in pH, a decrease in water-holding-capacity (WHC) and insoluble protein, and increased resistance of the

fibrils to low and high speed homogenization. This indicates cross-linking between protein molecules resulting in tougher, drier products. They concluded that the structural proteins of muscle are denatured but not proteolysed. A decrease in WHC can lead to an increased drip loss in meats. At a given pH, the irradiated meat has a lower WHC than the non-irradiated meat, a similar phenomenon as in heat denatured meats (Hamm and Deatherage, 1960).

In contrast to the contraction of the fibrils and a more rigid structure as previously mentioned, increased tenderness of irradiated meat was reported at high dosage levels. Bailey and Rhodes (1964) observed increased solubility of collagen in irradiated beef muscle and related this to tenderization of meat.

Whitehair et al. (1964) compared a high-temperature short-time blanching (HTST) to that of low-temperature long-time blanching (LTLT) used in enzyme inactivation of meat. They found that meat was more tender in irradiated samples with HTST, but showed no significant effect of irradiation on tenderness by LTLT.

### **Effect of Heating on Quality of Meats**

The heating of meat is accompanied by changes in flavor, texture, color, aroma, and nutritive value. The method of heating and its severity largely determines the effect on meat constituents particularly on meat proteins. When muscle proteins are exposed to heat, several changes occur such as shrinkage and hardening of tissues, release of juice and discoloration. In general, protein denaturation and protein coagulation with changes in solubility are noted. The rate

of cooking also affects the quality of the meat. Meat cooked quickly to a given temperature has a lower cooking loss and is juicier than meat that is cooked slowly to the same temperature (Bramblett and Vail, 1964).

### **Tenderness and Juiciness**

Different categories of muscle proteins yield different effects on meat palatability upon heating. Heat-induced changes on myofibrillar and stromal tissues have effect on tenderness and WHC or juiciness of meat. In general, cooking makes connective tissues more tender by converting collagen to gelatin while it toughens proteins of the myofibrils (Lawrie, 1985). Elastin, a rubber-like protein that is present in small amount in connective tissues, shrinks and hardens upon heating (Laakonen, 1973). Collagen, a major component of connective tissues, undergoes some physical changes that cause an increase in solubility and turns soft (Machlik and Draudt, 1963). These conflicting influences help to explain why different muscles react differently to cooking.

Effect of heating on tenderness of meat increases as the degree of heating is increased. *Longissimus dorsi* (low connective tissues) is tender, and *biceps femoris* (high connective tissues) is tough when boiled at 61°C, but the reverse holds true when they are braised at 100°C (Cover and Hostetler, 1960). Solubility by heat increases gradually from about 60°C to 98°C when the conversion to gelatin is high at higher temperatures (Paul et al., 1973).

Draudt (1972) studied the effect of heat-induced changes on tenderness and WHC of meat. Heating up to 40°C does not remarkably affect the mechanical

properties of meat. Heating in the temperature range of 50 to 60°C, led to collagen shrinkage without appreciable solubilization. Machlik and Draudt (1963) reported collagen shrinkage and its conversion into a more soluble form. Exposure of beef to temperatures up to 50°C had little effect on meat tenderness. However, at a temperature of 57°C, the tenderness of meat increased slowly, and at 64°C the tenderness reached a maximum. These changes are thought to be caused by collagen shrinkage which occurred at 60°C. At 73°C, rapid shrinkage of collagen was followed by protein hardening and toughening. Continued heating at this temperature resulted in substantial hydrolysis of collagen and eventually, caused meat tenderization.

Davey and Gilbert (1974) studied the effect of cooking temperature on shear force value, shrinkage, and weight loss on beef. They found the shear force value to increase approximately three fold at the end-point temperature of 40 to 50°C and 4 to 6 fold at 65 to 75°C. Changes in the first phase corresponded with denaturation of actomyosin and sarcoplasmic proteins. In the second phase, there was collagen shrinkage resulting in the loss of meat juices. A minimum shear force which corresponded to a maximum tenderness was found between end-point temperatures of 50 to 60°C (Draudt, 1972; Schmidt et al., 1970) due to the shrinkage and fragmentation of connective tissue (Schmidt and Parrish, 1971).

Heating to the end-point temperature of 30 to 50°C causes most decreases in WHC of meat (Bouton and Harris, 1972). This is a temperature range which the myofibrillar proteins coagulate and it corresponds to the first phase of toughness

reported by Davey and Gilbert as above. Following heat denaturation of proteins, the myofibrillar network tightens which results in the release of meat juices (Hamm, 1960) and rigidity of tissue (Robert and Lawrie, 1974).

Tenderness and juiciness are closely related; the more tender the meat the more quickly the juices are released by chewing, and the more juicy the meat appears (Bratzler, 1971). The principal sources of juiciness as detected by consumers are intramuscular lipids and water (Judge et al, 1989). However, little research shows a strong positive influence of lipid components in tissues (marbling) on tenderness (Judge et al, 1989). Marbling in meat may enhance juiciness in an indirect way. During cooking, melted fats translocate along bands of perimysial connective tissues. This distribution of lipid may act as a barrier to moisture loss during cooking (Judge et al, 1989). In spite of influences of fats, the major contributor to the sensation of juiciness is water remaining in cooked product.

Juiciness in cooked meat has two organoleptic components. The first is the impression of wetness during the first few chews and is produced by the rapid release of meat fluid. The second is the feel of sustained juiciness resulting from the slow release of serum and stimulatory effect on salivary flow (Bratzler, 1971). Lean red meat contains approximately 75% water, of which 4-5% is electrostatically bound to either myosin or actin depending on the shape and charge of protein (Hamm, 1969). Myofibrils are well suited to retain water because the three-dimensional network of their filaments provides an open space for water to be immobilized (Wismer-Pedersen, 1971). Tightening this space between the

myofibrillar network by contraction or protein denaturation, results in a decrease of immobilized water and an increase in expressible water (Hamm, 1969). Marshall et al. (1960) found that during roasting beef, as the internal cooking temperature increased, evaporative losses also increased. Heating of muscle tissues causes denaturation of myofibrillar proteins followed by coagulation, shrinkage of the myofilaments, and a tightening of the microstructure of myofibrils (Cheng and Parrish, 1976). All these can increase the amount of free water in muscle tissue and is evidenced by increased cooking loss percentages (Cross et al., 1986). As a result, when the internal temperature of the meat increases, the meat becomes less juicy. Hosteller and Landmann (1968) found that when meat is cooked to 53°C or higher, myofibrillar proteins lose their WHC through coagulation. The immobilized water in the proteins of the myofilaments escapes from the inter-myofibrils together with some soluble sarcoplasmic proteins.

### **Flavor and Aroma**

Flavor and aroma are terms used to describe senses of taste and smell. Taste is defined as the sensory attribute of certain soluble substances perceptible by the taste buds, usually of the four sensations: sweet, sour, salty, and bitter (Cross et al., 1986). Odor represents the sensory attribute of certain volatile substances perceptible by the olfactory organ when these volatile materials stimulate nerve endings in the lining of the nasal passages (Cross et al., 1986; Judge et al., 1989). Flavor is a complex combination of the olfactory (smell) and gustatory (taste) attributes perceived during eating (Cross et al., 1986). It is

influenced by tactile, thermal, pain, kinesthetic effects (Cross et al., 1986), texture and pH (Lawrie, 1985). It is thought that the perception of flavor is the action of odor and taste substances with enzyme-catalyzed reactions in the olfactory receptors and taste buds respectively (Haagen-Smit, 1952). The active part of a taste or odorous molecule fits some part of a protein structure in the olfactory receptors or taste buds, thereby altering reactions and giving rise to characteristic odors and flavors (Haagen-Smit, 1952). There appears to be a one to one relationship between the frequency of molecular vibration of the stimulating odoriferous compounds and the properties of the corresponding response of the olfactory bulb (Wright et al., 1967). Under ideal conditions, the sense of smell is approximately 10,000 times more sensitive than that of taste (Lawrie, 1985). This sensitivity of olfactory receptors accounts for the innumerable odor sensations a human is able to perceive, as opposed to taste sensations.

The flavor of raw meat is described either as a weak salty, serum or blood-like (Bratzler, 1971), and a lactic acid taste (Cross et al., 1986). The "serum" flavor of raw meat or beef steak is due to a combination of blood salts and salvation, which, after cooking is described as brothiness and mouthfulness (Bratzler, 1971). The true meat flavor develops at the heating temperature exceeding 70°C (Cross et al., 1986). The persistence of this "mouthfulness" during chewing is referred to as "bouquet" and may extend into the "aftertaste" (Bratzler, 1971). Pork has a "mouth-coating" flavor rather than a "mouth-filling" character. The ultimate flavor and aroma of meat is influenced by the cooking method, the kind and cuts of meat, and the

treatment of the meat before cooking (Bratzler, 1971).

More than 600 volatiles are known in beef (MacLeod and Seyyeddin-Ardebili, 1981), however, the contribution of these to flavor development is poorly understood (Judge et al., 1989). These volatiles include sulfur and nitrogen compounds, hydrocarbons, aldehydes, ketones, alcohols, and acids (Judge et al., 1989). Heat induced reactions that lead to the production of meat flavors include the pyrolysis of peptides and amino acids, the degradation of sugars, the oxidation, dehydration, and decarboxylation of lipids.

Most constituents responsible for meaty flavor are water-soluble components of muscle tissues (Judge et al., 1989) that contain inosinic acid and a glycoprotein, yielding a meaty odor upon heating (Batzer et al., 1960). Extracts of raw beef yield a recognizable boiled beef aroma upon heating. They contain methionine, cysteic acid, and 2-deoxyribose (Mabrouk et al., 1967). When cysteine (or methionine) was heated with ribose, a pork-like aroma was developed. When other amino acids were included, an aroma closer to that of beef resulted (Lawrie, 1985).

Hornstein (1971), concluded from several studies, that protein per se contributes little to meat flavor but that amino acids and reducing sugars are important precursors. Lipid studies indicate that fat may affect meat flavor in two ways. First, on oxidation, fatty acids produce carbonyl compounds that are potent flavor contributors. Second, fat may also act as a storage depot for odoriferous compounds that contribute to species difference in flavor and aroma. Amino acids sequence (Lawrie, 1985), as well as the carbohydrates of meat are important in

producing flavor on heating (Fagerson, 1969). Volatiles in heated meat include ammonia, acetaldehyde, acetone and diacetyl, fatty acids, dimethyl sulfide, hydrogen sulfide (Yueh and Strong, 1960). The concentration of reactive sulfur compounds increase with prolonged heating (Persson and Von Sydow, 1973). The incorporation of sulfur into heterocyclic ring systems such as thiazoles (Schwimmer and Friedman, 1972) and pyrazines (paradiazines) (Koehler and Odell, 1970) is common with progressive heating.

### **Color**

Color is probably the most obvious indicator consumers use when purchasing meat. From experience, consumers expect fresh cuts of beef to be bright red and pork to be greyish pink in color. Pigments in meat consist largely of two proteins: hemoglobin, the pigment of blood, and myoglobin, the pigment of muscle. In well bled muscle tissue, myoglobin constitutes 80 to 90 % of the total pigment (Judge et al., 1989). The color of the meat surface depends, not only on the quantity of myoglobin but also on the type of myoglobin molecule, on its chemical state, and on the chemical and physical condition of other components in the meat (Lawrie, 1985).

Myoglobin quantity varies with the species, age, sex, muscle, and physical activity (Lawrie, 1985). In general, beef and lamb have more myoglobin than pork (Judge et al., 1989). In the same species, a more mature, active, male animal would contain more myoglobin content than a young, less active, female counterpart. The constantly operating muscle of the diaphragm has more

myoglobin than the less intensive used *longissimus dorsi* (Lawrie, 1985).

Myoglobin (MW 16,000) is a conjugated protein consisting of an iron porphyrin, heme, attached to a simple globin protein. The heme iron functions to bind oxygen while the globin protein serves to surround and protect the heme. The hemoglobin (MW 67,000) molecule can be thought of as four myoglobin molecules bound together. In myoglobin, six bond orbitals of iron are present. The iron atom is bound to four nitrogen atoms in the porphyrin ring and one nitrogen atom of a histidine molecule of the globin protein. The sixth bond orbital is open for the formation of complexes with several compounds (Bodwell and McClain, 1971). Variations at this position are, in part, responsible for differences in the color of meat (Cross et al., 1986).

Changes in meat color are due to two chemical reactions with the heme portion of myoglobin. One reaction is dependent upon the molecule bound at the free binding site of the heme molecule. The other involves the oxidation-reduction state of the iron molecule in the heme (Cross et al., 1986). Pigments of raw meat consist of deoxymyoglobin ( $\text{Fe}^{+2}$ , deoxygenation form, purplish-red), oxymyoglobin ( $\text{Fe}^{+2}$ , oxygenation form, bright red), and metmyoglobin ( $\text{Fe}^{+3}$ , brown) (Watt et al., 1966). In living tissue, the reduced form of myoglobin exists in equilibrium with its oxygenated form, oxymyoglobin. This oxygenation reaction produces meat in red color and is called blooming (Clydesdale and Francis, 1971). Oxymyoglobin will dominate within 30 to 40 minutes of full exposure to air. If only small quantities of oxygen are present, such as in a partial vacuum or a sealed semi-permeable

package, the heme iron becomes oxidized. In this oxidized state of myoglobin, the pigment is brown (Judge et al., 1989). The oxidized state of myoglobin can be reverted to the reduced state via enzyme activity in the presence of reducing substances such as NADH (Cross et al., 1986).

When meat is heated, the globin moiety of myoglobin denatures; thus, its function of protecting the heme diminishes. This results in the oxidation of heme iron, a change of molecules at the free binding site, and metmyoglobin predominates (Cross et al., 1986). These authors stated that myoglobin and other sarcoplasmic proteins denature in the range of 40 to 50°C. Bernofsky et al. (1959) studied the effect of temperature on denaturation of myoglobin from beef as measured by decreased water extractability. They found that the denaturation of myoglobin in meat is higher than in the solution. Below 65°C, myoglobin denaturation may arise from enzymatic action or co-precipitation rather than from temperature.

Other authors described the occurrence of cooked brown pigments as the mixture of pigments. The main component is the denatured myoglobin compound, globin nicotinamide hemichrome (ferric form) (Bodwell and McClain, 1971) or globin myohaemichrome. (Lawrie, 1985). In beef, the greyish brown color develops when the internal temperature of meat reaches 70 to 80°C (Jensen, 1949).

Other factors contributing to the brown color of cooked meat include the caramelization of carbohydrates and Maillard reactions between reducing sugars and amino groups (Lawrie, 1985). The latter is particularly marked in pork which is

high in reducing sugars at post-mortem (Sharp, 1957) and low in myoglobin concentrations (Pearson et al., 1962).

### **Combination Treatments of Radiation and Heat**

The main objective of radiation preservation of food is to destroy pathogenic microorganisms (Urbain, 1986), and also reduce the number of the spoilage microflora. Certain foods, such as pork, have a threshold dose of gamma radiation as low as 1.75 kGy (Sudarmadji and Urbain, 1972). When ionizing radiation is applied to a food medium that facilitates accessibility of radiation-induced-radicals to react, undesirable flavor changes may develop (Urbain, 1986). The combined treatment of radiation to other physical or chemical agents may involve synergistic or additive action of the combination processes. The combination treatment may decrease the severity of either treatment when applied alone and in turn, may result in cost and/or energy saving. Moreover, it may bring about improvements in the sensory properties and bacteriological quality of the foods.

Since irradiation is a process involving energy input, the combination of radiation with another energy source seems logical (Urbain, 1986). Heat and radiation appear to have the same inactivation mechanism to target molecules, i.e. DNA and enzymes. When applied separately, the order in which heat and radiation are applied seems to be significant in destroying microorganisms.

Kempe (1959) and Kempe et al. (1959) reported that *C. botulinum* spores and *C. sporogenes* (P.A. 3679) spores were easily destroyed with heat following

irradiation. The complementary effect was found to increase with the irradiation dose.

Anderson et al. (1967) applied heat before, during, and after irradiation and concluded that mild heat treatment (75 to 87.5°C) following irradiation reduced the survival of spores of *C. botulinum* strain A-5 appreciably. In addition, heat applied before and during radiation actually increased the radiation resistance of the spores, especially in the temperature range of 80 to 87.5°C.

Huber et al. (1953) showed the synergistic effect of heat and electron radiation in milk. In this case, heating just prior to irradiation seemed to be considerably more effective than heating after irradiation. They obtained sterile milk with 30 seconds of heating at 73.5°C followed by irradiation of 4.65 kGy. The flavor of the sterile milk was only slightly below that of un-inoculated fresh control.

Kiss and Farkas (1981) studied the combined effect of gamma irradiation and heat treatment on the microflora of spice extracts. The authors irradiated their samples aerobically at the dose levels of 0, 1.6, 2.4 and 4.0 kGy at ambient temperature, followed by the heat treatment at 80, 90, and 95°C. They found that the heat resistance of the surviving mesophilic microflora of irradiated spice extracts was significantly decreased. The heat sensitization effect of radiation tended to increase as the dose increased.

Grecz et al. (1981), applied radiation at 0.5 and 1.0 kGy followed by heating at 90°C for 10 and 30 min to *Bacillus subtilis* 168 spores. Among the four possible combination of treatments, they observed a synergistic effect to be highest (5.72 log

reduction due to synergism) at the combination treatment of 1.0 kGy and 90°C/30 min. A mild synergism (1.22 log reduction) was observed at the combination treatment of 0.5 kGy and 90°C/30 min. No synergism was observed, at the other two combination of treatments, using irradiation at 0.5 or 1.0 kGy followed by heating at 90°C for 10 min. The amount of single strand breakage (SSB) of DNA was also reported and the percentage difference between computed and observed SSB were summarized. Radiation at 1 kGy followed by heating at 90°C/30 min showed the highest percentage difference in SSB of 85%.

Effect of irradiation followed by heating on vegetative bacteria was studied using X-rays. A synergistic effect was found in cases of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas fluorescens* while an additive effect was found in *Salmonella typhimurium* (Szcawinska, 1981).

Most of the work on combination treatment of heat and radiation has been performed as two-step processes. No general valid statement can be extracted from the literature regarding the significance of the time interval between individual treatments (Farkas, 1990). In *Bacillus subtilis* spores, the radiation-induced heat sensitivity persisted for at least two months in dry wheat flour (Farkas and Andrassy, 1981).

Simultaneous heating and radiation (thermoradiation) has been less investigated as compared to the two-step processes. Thermoradiation (55 to 65°C/30 kGy gamma) was used for the commercial scale sterilization of catgut in the former Union of Soviet Socialist Republics (USSR) from 1973 to 1982 (Woods and

Pikaev, 1994). This is probably the only practical application of simultaneous heating and radiation to control microorganisms to date. Grecz et al. (1967) studied the difference in radiation resistance of spores at different irradiation temperatures from -196 to 95°C. They found that the radiation resistance of spores of *C. botulinum* strain 33A was strongly affected by the temperature during irradiation. Very low radiation resistance was consistently observed at 0°C. Below 0°C the resistance of spores increased. The solidly-frozen medium caused a decrease in diffusion of molecular fragments and free radicals and thus the indirect bactericidal effect of radiation was reduced. It should be noted that, as the temperature increased above 0°C, the resistance of the spores also increased. Spore resistance was highest at 70 to 75°C and dropped sharply at temperatures above 80°C. However, Grecz et al. (1971), statistically computed a linear change in D-values for spores of *C. botulinum* 33A in canned ground beef with irradiation temperatures from -196 to 95°C. As the temperature of irradiation was increased from -196 to 65°C, the corresponding radiation D-value decreased linearly without a sharp drop in D-value at 0°C. From 65 to 85°C there was a rapid drop in D-values with a very sharp drop in the temperature range of 85 to 95°C. Whether the drop in radiation resistance of spores was caused by a synergistic effect of heat and radiation, or by a simple additive effect of the two parameters, was not determined.

Grecz et al. (1977) studied the characteristic pattern of radiation resistance of *C. botulinum* strain 33A spores as a function of temperature during radiation. Survival curves of the spores suspended in broth and irradiated under oxygen and

nitrogen atmosphere were plotted against the temperature (-10 to 90°C) of irradiation (8 kGy). Lowest radiation resistance was observed at 0°C and peak survival curve at around 50°C. The authors explained that the pattern of survival curves followed a thermorestitution pattern (annealment of lethal radicals).

Thermorestitution characteristic has also been observed by other authors (Webb et al., 1958; Grecz et al., 1967). These authors reported that irradiation during elevated but sub-lethal temperatures (50-70°C) may actually enhance the survival of certain bacterial spores. Anellis et al. (1973) found a similar pattern of radiation sensitivity in *Streptococcus faecium* A<sub>21</sub> in phosphate buffer. D-values of *S. faecium* decreased as temperatures of irradiation decreased (-196 to +5°C) with a sharp drop at around 0°C. Radiation sensitivity of bacteria at higher temperatures was not determined.

Fisher and Pflug (1977) observed synergism of wet-heat thermoradiation of *B. subtilis* var. *niger* spores over the temperature range of 75 to 90°C. They stated that no synergism is possible unless each physical treatment reaches its lethal range. By calculating the energy of activation, they concluded that, maximum synergism occurred under conditions where heat and radiation individually destroyed spores at an approximately equal rate.

In a recent paper, Thayer and Boyd (1995) inoculated *longissimus dorsi* of beef with mixed strains of *L. monocytogenes* and the effect of gamma irradiation on the survival of this pathogen at -60 to +15°C was studied. They found that the radiation sensitivity increased as temperatures of irradiation increased, with a sharp

increase of sensitivity at  $-5^{\circ}\text{C}$ . The survival curve showed a trend toward thermo-restoration phenomenon up to the temperature of  $+15^{\circ}\text{C}$ . It was not possible to conclude whether the survival curve actually showed the thermorestitution effect as radiation sensitivity at higher temperatures was not included in the experiment.

### **Sources for Food Irradiation**

The first observation that there were unknown forces at work within the atom was with the discovery of radioactivity by Henri Becquerel in 1896. It was Marie Curie, a Polish scientist and two time Nobel Prize winner, who later named this new phenomenon 'radioactivity', a name we still use today (Marion and Hornyak, 1985). Since then, the development of nuclear physics has been connected with the findings of subatomic structure, the emergence of the concepts of quantum theory, the discoveries of nuclear fission and fusion, and accumulation of other remarkable discoveries which lead to a fundamental understanding of nuclear science.

Ionizing radiation (X-rays) was first applied to control *Trichinella spiralis* by B. Schwartz in 1921. Wust obtained a French patent on preservation of foods by irradiation in 1930 to destroy bacteria on foods (Goresline, 1983). However, it was not until the time of World War II in the 1940's that sources to irradiate foods became commercially available (Urbain, 1986). In 1943, X-ray sterilization of hamburgers was investigated by Professor B. E. Proctor at the Massachusetts Institute of Technology (Rosenthal, 1992), and in 1945, the radiation preservation of fish and related products at the Tokyo University of Fisheries (Kawabata, 1981).

The first electron irradiation was a Capacitron which delivered high doses and penetrated to a depth of 12 mm in water (Goresline, 1983). Seafoods, dairy products, vegetables, and fruits were reported to have been effectively sterilized with these fast electrons without impairment (Brasch and Huber, 1947). The understanding of how atoms work through the basic knowledge of nuclear physics, radiation chemistry, and modern biology have led to the selection of X-rays, gamma rays, and electron beams for food irradiation.

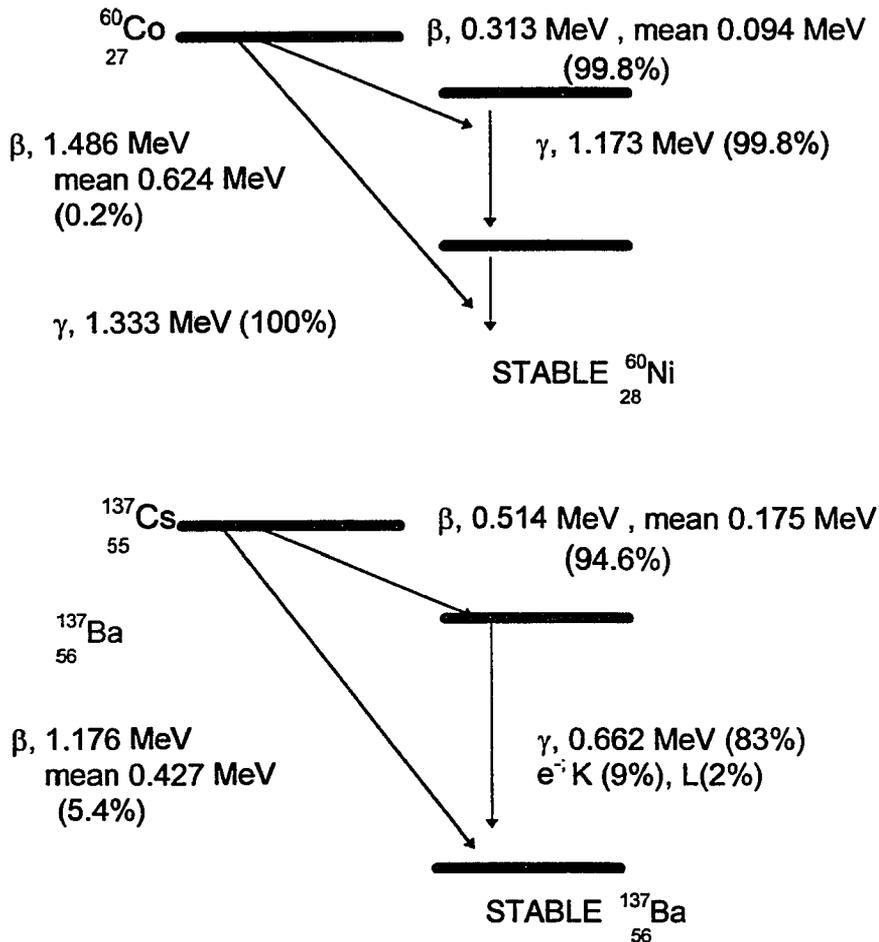
These ionizing radiations were chosen because of their ability to practically penetrate through foods and packaging materials. At a maximum safe limit of energy, i.e., 5 MeV for photons and 10 MeV for electrons, they do not induce radioactivity in foods (FAO/IAEA/WHO, 1981). Other ionizing radiations such as alpha particles have too low a penetrating power to be useful while neutrons may induce radioactivity.

In developing the nuclear technology to preserve foods, it is essential to minimize any undesirable effects or changes which can result from the process. There are several radionuclides which could serve as gamma ray sources for food but the most suitable ones are from cobalt-60 and cesium-137. These are the only radionuclides approved for food irradiation. Cobalt-60 is made from highly purified, natural non-radioactive cobalt-59 bombarding with neutron radiation. Cobalt-60 decays at the half-life of 5.272 years emitting gamma rays with energies of 1.173 and 1.333 MeV and eventually becomes non-radioactive nickel 60 (Rosenthal, 1992). Cobalt-60 decays predominantly by emissions of a 0.313 MeV beta particles

to give an excited state of nickel-60. Radioisotope nickel-60 loses energy by emitting two gamma photons in cascade with energies of 1.173 and 1.333 MeV. In the fabrication of the source, cobalt-59 is generally irradiated in the form of pellets, small slugs, or thin disks of metal to give a uniformly active material (Woods and Pikaev, 1994). These are placed in containers that are then assembled into radiation sources of the desired size and activity. The walls of the metal containers serve to filter out the low penetrating beta-radiation emitted by the cobalt-60. In food irradiation, the activity of cobalt-60 sources may vary from thousands of curies, for research purposes, to larger sources in the order of hundred of thousands to more than one million curies in multipurpose radiation processing facilities.

Cesium-137 is a fission product from uranium and other elements in a nuclear reactor (Rosenthal, 1992). This radioisotope decays by emitting two levels of energies. The beta particle of 1.176 MeV (5.4%) give rise to the ground state of barium-137 and another level of beta-particle of 0.514 MeV (94.6%) to yield an energy rich metastable form of barium-137. The metastable barium decays to the ground state of the nucleus with a half life of 2.55 min by emission of a 0.662 MeV gamma photon (83%) or emission of a conversion electron (11%) whose energy is 0.662 MeV less the binding energy of the electron in the atom (Woods and Pikaev, 1994). With barium-137m, about 9% of the decay events give conversions electrons (0.625 MeV) whose origin is the K-shell of the atom and about 2% conversion electrons (0.656 MeV) that originate from the L-shell of the atom (Woods

and Pikaev, 1994). Radioactive decay of the gamma emitting isotopes cobalt-60 and cesium-137 is summarized by the same authors, as follows:



There are practical reasons for the preferred use of cobalt-60 as an irradiation source. These include a greater degree of overall efficiency, better penetration by gamma rays, and greater environmental safety due to the insolubility of metal cobalt in water (Jarrett, 1983). Cesium-137, a fission product of nuclear reactors, is available as cesium chloride which is water soluble.

X-rays were one of the first irradiators developed for food irradiation in the early pioneering time. Bremsstrahlung X-rays are generated by placing a converter plate of high atomic number metal in the path of electrons within an evacuated tube (Rosenthal, 1992). When fast electrons hit matter, they lose energy by collision with electrons of the target material and by deflection via the electric field of the nucleus. Bremsstrahlung X-rays are produced whenever high -speed electrons are rapidly decelerated, in this case, by passing through the electric field of an atomic nucleus, leading to their description as braking radiations (Woods and Pikaev, 1994). Bremsstrahlung X-rays production is an alternative means of energy loss by fast electrons to the production of ionization and excitation. They are favored at high electron energies and by high atomic numbers of stopping materials (Urbain, 1986; Spinks and Woods, 1990; Woods and Pikaev, 1994).

When the energy supplied to an atom is equal or greater than the ionization energy, it is possible to remove one of the inner electrons, especially those in K-shell. Once there is a vacancy in the K-shell, it is energetically more favorable for an electron in the outer shell to occupy the K-shell vacancy. It is most likely that an L-shell electron will make this transition, emitting an energetic, characteristic X-ray in the process. These characteristic X-rays contain energies specific for the element bombarded (Rosenthal, 1992). Following collision, they superimpose on the emitting Bremsstrahlung X-rays which appear as electromagnetic radiation of a wide and almost continuous range of wavelengths. In the case of a high atomic number metal, eventually there will be a cascading of electrons and the emission of

a series of X-rays. These X-rays have more penetrating power than the initial electron beam but the conversion process has a low energy of about 5-7% for 5 MeV electron (Woods and Pikaev, 1994; Spinks and Woods, 1990).

The fraction of the electron energy converted to X-rays is governed by the initial electron energy and the atomic number of the stopping material (Urbain, 1986). Photons of X-rays and gamma rays react with matter in similar manners. In food irradiation, they are regarded as having similar characteristics although they are different in wavelengths and frequencies in the electromagnetic spectrum.

There are limitations by which electromagnetic waves are produced. At lower frequencies such as radio frequencies (RF) and microwaves, electronic methods have been used to generate these frequencies up to  $\sim 10^{12}$  Hz. For X-rays which have frequencies between  $10^{18}$ - $10^{19}$  Hz, atomic radiations are used to generate electromagnetic waves in the frequencies up to approximately  $10^{20}$  Hz. Gamma rays with higher frequencies of  $10^{21}$  Hz are produced within nuclei and by interaction involving very high speed particles (Marion and Hornyak, 1985).

Electron accelerators are the most widely used machine sources as compared to other machine sources that produce accelerated positive ions and bremsstrahlung X-rays. The advantages of employing electron accelerators lie in the fact that there is an extremely low probability of inducing radioactivity in the irradiated products, that the beams can be switched on and off, and that the electrons can be directed to the foods thus increasing efficiency. The energy of the electron beams can be designed to penetrate foods at a required depth.

Electrons distribute their energy to the absorber in a non-uniform pattern. As an electron travels through a medium, it gradually loses energy by transferring its energy to the atoms of the absorber and simultaneously slows down. As the velocity diminishes the number of interactions per unit distance traveled increases (Urbain, 1986). When the velocity becomes so small, the electron is captured by an appropriate entity of the absorber. Therefore, an electron pathway displays initially small energy transfer, then the density of energy transfer increases just below the surface of the absorber, and then the energy transfer stops. The non-uniform dose distribution has led to the concept of the useful range of electrons (Woods and Pikaev, 1994). The useful range can be increased by irradiating the material from two opposite sides. This gives a useful range of about 2.2-2.4 times greater than single-sided irradiation (Markovic, 1981).

Because the electron possesses a small mass and carries a negative charge, each time the electron approaches the target atoms, it is deflected from its path by orbital electrons and by positive atomic nuclei. For these reasons, electrons can penetrate into the medium less than photons. Gamma radiation with an energy approximately between 0.15 and 4 MeV will penetrate about 30 cm of water. In comparison, accelerated electrons with an energy of 10 MeV will penetrate only to a depth of about 4 cm (Rosenthal, 1992). Electrons with sufficient kinetic energy near the surface of foods may escape. However, at some point below the surface, there are some primary and secondary electrons that have inadequate energy to escape from the absorber. This is the region of maximum energy transfer, and located

somewhat below the surface of the absorber. At deeper regions, the beam intensity reduces until none of the energy remains as the depth increases and the limit of penetration is reached (Urbain. 1986).

### **Interaction of Photons and Electrons with Matter**

Photons refer to a quantum of electromagnetic energy which includes gamma rays and X-rays. At the high energy end of the electromagnetic spectrum lie gamma rays which are produced during changes inside the atomic nucleus. Gamma rays possess wavelengths in the range of 0.0005 to 0.01 nm which correspond to the energy levels of 120 keV to 2.5 MeV and approximately  $3 \times 10^{19}$  to  $6 \times 10^{20}$  Hz frequencies. Bremsstrahlung X-rays are produced by energy state positions of inner electrons close to the nucleus. They cover the wavelength in the order of 0.01-10 nm with the energy levels of 0.12 keV to 0.12 MeV and approximate frequencies of  $3 \times 10^{16}$  to  $3 \times 10^{19}$  Hz (Rosenthal, 1992).

Reactions of photons with matter involves transferring energy from photons to the orbital electrons and/or nuclei of the atoms. When a photon travels with energies below 0.1 MeV in absorbers having low atomic numbers, the photon gives all of its energy to an atom. This energy is used to overcome the ionization potential and an orbital electron is ejected. The remaining energy is acquired by the ejected electron and the residual of the atom in the form of kinetic energy. This interaction is called the photoelectric effect and can occur at the outer shell or inner most K-shell.

For photon energies greater than approximately 0.1 MeV, the Compton effect occurs. In this case, part of the photon energy is used to free the orbital electron and also in imparting kinetic energy to the ejected electron and to the remnant atomic ion. The photon continues traveling in a deflected direction with the remaining energy. The Compton effect is the most important mechanism of photon energy transfer in food irradiation.

Pair production occurs when photons carrying energy of at least 1.02 MeV are absorbed in the nuclei of the atom. Two particles, a positron and an electron are formed. The remaining energy imparted by the photons, if any, will be divided between the two particles and enable them to travel through the medium. Ionization and excitations can occur along their paths.

Charged particles such as electrons of moderate energy interact largely through the electric (Coulomb) forces between them and the orbital electrons of the atoms of the absorber (Urbain, 1986). In parallel to the interaction of photons and matter, energy equal or higher than the ionization energy is transferred to the orbital electrons, they are ejected from the atom. This process is called ionization. When the energy of the charged particles is less than the ionization potential energy it is transferred to the orbital electron. In this case, the electron is not ejected but vibrated, and/or rotated, a process called excitation.

In a complex system like food, ionizing radiation causes changes in each and every accessible molecule by direct interaction. The resulting radicals and remaining energy carried by radiation react further with secondary molecules. This

is called the indirect action of reactive particles.

Several authors (Urbain, 1986; Woods and Pikaev, 1994) have summarized the stepwise radiolytic mechanism that is completed within  $10^{-11}$  sec before diffusion takes place (steps 1 through 7) and the neutralization (step 8) as follows:

1. Initial deposition of energy creates spurs or columnar tracks containing ion pairs and excited molecules
2. Ion dissociation to yield radicals, molecules. There are also reactions of ions and molecules that yield more ions and free radicals
3. Production of solvated electrons by solvation of electrons and positive ions
4. Geminate (pairing) recombination of ions to yield excited molecules
5. Dissipating excitation energy without reaction, dissociation of excited molecules to radical products and molecular products
6. Recombination of caged radicals, diffusion takes considerably longer than the processes above, in the order of  $10^{-8}$  sec, or longer, after ionization
7. Following these steps, there is diffusion of radicals, molecular products, and possibly triplet excited states from the spur
8. After approximately  $10^{-10}$  sec. there is neutralization of ions to form molecules and also radical combination. Other types of reaction are also possible, e.g., reactions of radicals with oxygen

Ions may dissociate, take part in geminate ion-molecule reactions, undergo geminate recombination with an ion of opposite charge, or become solvated. Excited molecules may dissociate or dissipate their energy without reaction. Radicals may reform into the original material, or form dimers or unsaturated compounds. Irradiation reactions depend upon the conditions during irradiation and the composition of foods being irradiated. In foods that contain polar liquids such as water, solvation of the ions and diffusion of the solvated species predominate. In non-polar liquids such as hydrocarbons, geminate recombination of ions within the spurs predominates (Woods and Pikaev, 1994).

## **Rancidity and Its Measurement**

### **Definition and Nature of Rancidity**

The word 'rancid' is derived from *rancidus*, the Latin for 'stinking' (Sanders, 1989). Rancidity is the term used to describe the smell or taste of off-odor and/or off-flavor of food. Although certain off-flavors can develop in foods by reactions of carbohydrates or proteins, lipids contribute to a large portion of oxidation in meats.

Off-flavor in meat upon storage is often described as degree of rancidity. Three categories of rancidity can occur in foods, namely, oxidative rancidity, hydrolytic rancidity, and ketonic rancidity (Rossell, 1989). Oxidative rancidity of lipids, or sometimes referred to as autoxidation, is caused by reactions of oxygen with unsaturated fats. Autoxidation proceeds through a free radical chain reaction with the development of intermediates including hydroperoxides and peroxides. End

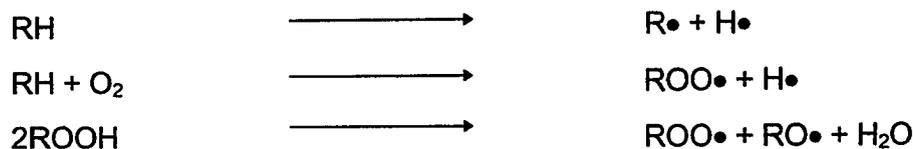
products of autoxidation are aldehydes and ketones. Hydrolytic rancidity refers to off-flavors produced by hydrolytic reactions of the triglycerides in the presence of moisture (Rossell, 1989) and enzymes with the liberation of the fatty acids (Hamilton, 1989). Hydrolysis usually takes place in the presence of lipase or esterase enzymes or microorganisms such as yeast, bacteria, or fungi. Ketonic rancidity occurs when fungi attack foods in the presence of limited amounts of water and oxygen. These reactions liberate a series of short-chain free fatty acids which are subjected to a  $\beta$ -oxidation. The end products consist of methyl ketones and aliphatic alcohols (Rossell, 1989). The first step of ketonic rancidity, the hydrolysis of fatty acids, is closely related to mechanisms of the hydrolytic rancidity.

Differences between them are end products. In the hydrolytic rancidity, a soapy odor is produced in contrast to a musty, stale odor in the case of ketonic rancidity.

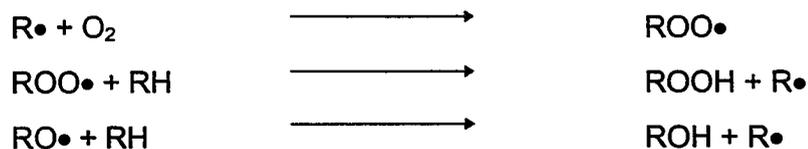
Oxidative rancidity is responsible for most of the off-flavor development in meat. It occurs as a two-step process 1) the formation of hydroperoxides, and 2) the formation of secondary products from the reaction of hydroperoxides (Chan et al., 1982). Reactions involve polymerization, fission, dehydration, and oxidation of hydroperoxides (Lea, 1962). End products are polymers of hydroperoxides, aldehydes, acids, keto-, hydroxy-, dihydroxy-glycerides, epoxide, that result in off-odors and flavors (Lea, 1962; Addis et al., 1983a). It should be noted that, hydroperoxides, the products from the first step, are relatively non-volatile and do not produce an off-flavor (Shahidi and Pegg, 1994).

Hydroperoxides, the intermediates in the autoxidation, may be formed by one of two mechanisms. The first route is the classical free radical mechanism which can occur in the dark. The second route is a photo-oxidation mechanism which is initiated by exposure of light to certain photosensitizer molecules (Hamilton, 1989). Formation of hydroperoxides by the free radical chain reaction route, proceeds by three phases of chemical reactions: initiation, propagation, and termination. In the initiation step, oxygen reacts with a hydrogen atom adjacent to a double bond (RH) connecting between carbon atoms, producing an alkyl radical ( $R\bullet$ ) and a hydroperoxide ( $\bullet\text{OOH}$ ). The propagation step is a series of reactions in which new free radicals are formed (alkyl  $R\bullet$ ; and peroxy  $\text{ROO}\bullet$ ) (Chan and Coxon, 1987). These two steps are parts of a self-propagating chain process that produces many hydroperoxide molecules. The self-propagating chain process can be stopped by termination reactions, where two radicals combine to give products that do not feed the propagating reactions (Nawar, 1985). Chain reactions are possible because of the low activation energies required. Peroxide formation steps require the activation energy of 4-5 kcal/mole and the free radicals combination step requires approximately 6-14 kcal/mole for the reaction to take place (Hamilton, 1989). The mechanism of free radical autoxidation of lipids is presented (Ho and Chen, 1994) as follows:

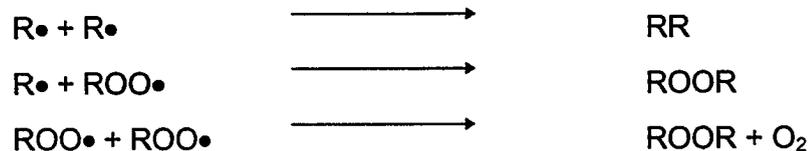
## Initiation: The formation of free radicals



## Propagation: The free-radical chain reactions



## Termination: The formation of nonradical products



(RH, R•, RO•, ROO•, ROOH represent an unsaturated fatty acid or ester, alkyl radical, alkoxy radical, peroxy radical, and hydroperoxide respectively)

Photo-oxidation has been recognized as an alternative to the free radical mechanism. It has been shown that different hydroperoxides are formed in the presence of light and certain photosensitizers (Yang and Min, 1994). Singlet oxygen has been suggested to be responsible for initiating lipid oxidation due to its ability to directly react with the electron-rich double bonds of unsaturated lipids (Korycka-Dahl and Richardson, 1978). The electron arrangement of triplet oxygen (two unpaired electrons) does not allow for a direct reaction of lipid molecules that exist in the singlet state (no unpaired electrons) (Ho and Chen, 1994). Singlet oxygen reacts approximately 1,500 times faster than triplet oxygen. It can be

produced by sensitizers, e.g. chlorophyll, myoglobin, erythrosine, riboflavine, and heavy metals (Hamilton, 1989).

Mammalian muscle is made up principally of water (65-80%), protein (16-22%), lipids (1.5-13%), non-protein nitrogenous substances (1.5%), carbohydrates (1.0%), non-inorganic constituents (1.0%) (Judge et al., 1989). Fat is probably the most variable component of meat and it varies from 9% in lean muscle to approximately 20% in retail fresh cuts (Sebranek et al., 1989). Lipids consist mainly of triglycerides and phospholipids. Most lipids are present in adipose tissue associated with the loose connective septa between the muscle bundles (marbling), between muscles (seamfat), and externally (sub-cutaneous) (Judge et al., 1989). PUFA's, the most susceptible sites to oxidation, exist in greater proportions in phospholipids than in triglycerides. Although total muscle is composed of  $\leq 2\%$  phospholipids, they are considered to be most responsible for oxidative rancidity in meat (Igene and Pearson, 1979).

Natural fats are glycerol esters of straight chain carboxylic acids of even number carbon atoms. Glycerol esters occur as mono-, di-, and triglycerides, with triglycerides predominate in fresh meats. The radicals of the triglycerides are composed of saturated or non-saturated fatty acids, with saturated and mono-unsaturated fatty acids predominate in meat fats (Dugan, 1978). Fatty acids having chain length of 10 carbon atoms or less are rarely found in animal fats. Fatty acids of 16-18 carbon atoms are more common, while those acids of 12, 14, and 20 carbon atoms present only in small quantities (Judge et al., 1989). The

predominant unsaturated fatty acids in animals are palmitoleic (C18:1, 2.2%), oleic (C18:1, 40.1%), linoleic (C18:2, 1.8%), linolenic (C18:3, 0.6%), and arachidonic acid (C20:4, 0.2%) (Judge et al., 1989). Oleic acid is the most abundant fatty acid in the animal body. Of the triglycerides in animal body fats, those that contain one palmitic fatty acid and two oleic fatty acids in each triglyceride molecule are most commonly found, followed by triglycerides containing one molecule each of oleic, palmitic, and stearic acid (Judge et al., 1989).

### **Measurement of Rancidity**

#### **Thiobarbituric acid**

The analytical measurement of rancidity, to be meaningful, should be correlated with consumer sensory scores. The thiobarbituric acid (TBA) assay has been recognized to be highly correlated with taste panel scores for oxidized and warmed-over flavors in muscle foods (Wilson et al., 1976). Sensory scores measure what the consumer actually perceives. Although sensory evaluation is the final measure of lipid oxidation in foods, it can only be used qualitatively. Chemical methods of rancidity measurement afford a more easily determined index of rancidity (Labuza, 1971).

The primary product formed by oxidation of lipids is a peroxide or hydroperoxide. Hydroperoxide from the primary reactions polymerize to form large molecular components not directly related to rancidity. Many breakdown reactions occur forming smaller compounds that are responsible for rancidity and are used to follow the development of oxidative rancidity (Evans, 1961).

Among several methods that measure the secondary products of autoxidation, thiobarbituric acid (TBA) test is the method frequently used. TBA reagent reacts with malonaldehyde to form a pink-colored complex that shows distinctive absorption maximum at 532 nm (Tarladgis et al., 1960). The concentration of this chromogen is used to evaluate the oxidative state of meats. The TBA test was once believed to be specific for malonaldehyde (Tarladgis et al., 1960; Tarladgis et al., 1964), but later data suggested otherwise. It was found that other aldehydes react with TBA to give red chromogens (Dahle et al., 1962). Baumgartner et al. (1975) observed that acetaldehyde and sucrose react with the TBA reagent to produce a complex with an absorption maximum at 532 nm. Other researchers have found that the TBA reagent reacts with other products of lipid oxidation to form red complexes with an identical absorption maximum as the TBA-malonaldehyde complex (Kosugi and Kikugawa, 1986; Kosugi et al., 1988). Malonaldehyde may also react with myosin (Buttkus, 1967), methionine and cysteine (Buttkus, 1969), citrulline, arginine, and 2-oxypyrimidine (Buttkus and Rose, 1972). Shin et al. (1972), observed the loss of activity of enzymes ribonuclease and papain in the presence of malonaldehyde. These reactions reduce the chance of malonaldehyde to react with TBA reagent leading to the decrease of TBA numbers after storage (Buttkus, 1967; Buttkus, 1969).

From the above observations, TBA numbers of meat tend to increase over the storage period, reach a maximum value, and then decline. During the early stages of meat flavor deterioration, significant correlations have been demonstrated

to exist between TBA value of meat volatiles and sensory scores (Shahidi et al., 1987; Ang and Lyon, 1990). Malonaldehyde contributes only to a small fraction of the overall end products of lipid autoxidation. Moreover, malonaldehyde has very little or no odor of its own thus raised a question about its validity as an indicator of lipid autoxidation (Shahidi, 1988). As a result, the term 2-thiobarbituric acid reactive substance (TBARS) is now commonly used in place of TBA number or value (Gray and Pearson, 1987). Although malonaldehyde represents only one of the TBARS, both share many similar properties and the reaction of malonaldehyde may be very close to those of TBARS. Patton (1974), suggested that TBA test results need to be interpreted with caution and should be compared with organoleptic evaluation or with findings by other suitable chemical tests. Addis et al. (1983b) advised that the TBA test be used to measure the extent of lipid oxidation, and not to quantify malonaldehyde, since numerous substances are known to be involved with the TBA reaction.

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**IRRADIATION AND HEATING EFFECTS ON D-VALUES OF *LISTERIA*  
*MONOCYTOGENES* SCOTT A IN GROUND PORK**

A paper to be submitted to the Journal of Food Protection

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**ABSTRACT**

Radiation resistance of *Listeria monocytogenes* Scott A in ground pork, packaged in air and under vacuum, was studied at the irradiation temperatures of 2°C (refrigerated) and 48°C (heated). More survivors of *L. monocytogenes* Scott A were found when cells were irradiated and recovered, under vacuum, than in air. Within the dose-range studied (0 to 1.5 kGy), irradiation of ground pork at 48°C resulted in overall less survivors than when irradiation was carried out at 2°C. The rate of listerial destruction, as determined by the reciprocal of the slopes of survival

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curves; however, appeared to be greatest for samples that were air-packed and irradiated at 2°C. D-values of *L. monocytogenes* Scott A were found to be 0.31, 0.35, 0.37, 0.42 kGy for refrigerated/air, heated/air, refrigerated/vacuum, and heated/vacuum treatments respectively. An overall cell injury was found to be 3.7%. Healthy and injured cells appeared to respond to irradiation in a similar pattern. When irradiation was performed at 48°C, the rate of listerial destruction followed the first order of kinetic and exhibited straight lines on semi-logarithmic plots. *L. monocytogenes* inoculated in ground pork and irradiated at 2°C appeared to exhibit shoulders on its survival curves.

## INTRODUCTION

Listeriae is a non-fastidious microorganism. It can grow over a wide range of temperature, pH,  $a_w$  and thus is ubiquitous in nature including food for human consumption. It is considered to be one of the newly emerged human pathogens although the first human listeriosis was reported as early as 1929 (Jay, 1992). The United States Department of Agriculture (USDA) has established a zero tolerance in 50 g of food for *L. monocytogenes* (Farber, 1993). However, total elimination of the organism from all foods may be impossible (Farber, 1993). Although listeriae has never been proven to be a causative agent for human listeriosis in meat, it has been linked to the consumption of refrigerated, un-reheated hot-dogs that caused listeriosis (Schwartz et al., 1988). The incidence of listeria in meat, including ready-to-eat meat, and the psychrotrophic nature of the organism make it a threat in

refrigerated foods.

The joint International Atomic Energy Agency/Food and Drug Administration/World Health Organization (IAEA/FAO/WHO) recommended that foods, irradiated below 10 kGy, are safe for human consumption and that wholesomeness tests are not required (WHO, 1981). This report has attracted the utilization of a mild irradiation process to control microorganisms in foods, a process called radurization. Conventional food processing often requires severe treatments to control pathogens which may cause undesirable changes in meat. Irradiation above the threshold level results in 'irradiated' flavor in meats and meat products which is considered undesirable by many consumers. Heating changes the appearances of meat and may destroy heat-sensitive vitamins. Packaging in oxygen-permeable film increases the rate of autoxidation in meat.

Among the several approaches to control human listeriosis is the use of hurdles or barriers to control the growth or to inactivate listeria in foods. These are combination of mild treatments, which, when applied singly, are not sufficient to control pathogens. The treatments when applied together, however, may work synergistically or additively to control pathogens. Irradiation of foods at sterilization doses has been a topic of research work for over four decades while irradiation at lower doses has been investigated within the past fifteen years. Not enough data is available for psychrotrophic pathogenic bacteria on their sensitivity to irradiation, particularly in combination with other parameters.

The purpose of this study was to test the hypothesis that irradiation was more effective to destroy pathogens when applied at a higher temperature (48°C) as compared to lower temperature (2°C). The second goal was to achieve the best combined treatment to control psychrotrophic pathogenic bacteria, i.e. *L. monocytogenes* in ground pork by combination effects of heating, irradiation, and vacuum packaging.

## **MATERIALS AND METHODS**

### **Preparation of Pork**

Frozen ground pork was obtained from the Meat Laboratory, Iowa State University and thawed in the refrigerator (4°C/48h) before use. Approximately 100 g of ground pork were formed into patties of 2 cm thick by 10 cm diameter by hand with the aid of a molder ring. Patties were divided into 4 identical portions of approximately 25 g each and individually placed in air or vacuum packages. Half of these samples were inoculated, in the geometrical center, with *L. monocytogenes* Scott A cells while the other half served as un-inoculated controls. Polyethylene packages and vacuum packages, with oxygen barrier property of 0.46-0.62  $\text{cm}^3/1\text{m}^2/\text{atm}/20^\circ\text{C}$  at 0%RH/24h, represented oxygen permeable and oxygen impermeable packages respectively. The inoculated, as well as the un-inoculated subgroups of samples, were packaged either in air or under vacuum (Fresh Vac. Model A300, CVP Systems Inc., Downers Grove, IL) at 380 torr. Samples were

subjected to different combination treatments of heating and/or irradiation (Figure 1).

### **Treatment by Temperature**

Refrigerated samples were held in the cold storage overnight to ensure the internal temperature of 2°C. The heated samples were placed in a 2 L water bath, preheated to 45°C, in a microwave oven (Radarange, Amana, IA). The heating process was stopped when the final temperature of pork patties reached 48°C. In each trial, extra patties were treated in a similar manner as controls. The temperature change of the pork was measured with a Thermistor Digital Temperature Tester to  $\pm 0.2\%$  (Hantover, Kansas City, Mo) with a heavy-duty meat probe. After the cooling and/or heating processes, pork samples were arranged, in a single layer, in insulated card-board boxes to prepare for irradiation. At the end of the treatments, samples were kept in a refrigerator (4-5°C) until the recovery procedure was completed, usually within 10 h.

### **Irradiation**

Ground pork was irradiated at the Linear Accelerator Facility of the Meat Laboratory, Iowa State University, at refrigeration temperature and at high temperature in insulated card-board boxes. Temperature changes of ground pork before and after irradiation were recorded. During irradiation, the temperature of refrigerated samples was found to be between 4 and 5°C, and that of the heated samples was between 45 and 46°C. Doses of 0, 0.5, 1.0, and 1.5 kGy were achieved by adjusting the Linac power level (1 or 3 kW) and the conveyor speed

(m/min). Absorbed dose was measured by placing 99% pure alanine pellets (Bruker Instruments Inc., Billerica, MA) on the upper and the lower surfaces of the pre-packaged patties and the average dose used. An Electron Paramagnetic Resonance instrument (EMS 104, Bruker Analytische Messtechnik, Karlsruhe, Germany) was used to determine the free radicals formed and thus, the absorbed doses. The instrument was calibrated with standard dosimeters traced to the national standard at the National Institute of Standard and Technology (NIST).

### **Proximate Analysis and pH Measurements**

Protein analysis was performed using the combustion analyzer instrument (Leco FP 428, Leco Corp., St. Joseph, MI) that has been standardized with EDTA (ethylene-diamine tetra acetic acid, Leco Corp.). Percent crude protein was calculated from nitrogen (percent) x 6.25. Fat content was obtained from n-hexane extraction of 5 g samples using a Soxhlet apparatus. Moisture content was analyzed by drying meat samples in the Gravity and Mechanical Convection Constant Temperature Cabinets (GCA, Precision Scientific Model 27, GCA Corp., Chicago, IL). The weight difference, of the meat before and after drying, was calculated to obtain the moisture level. The pH value was measured from the filtered slurry of ground pork (10 g) diluted with distilled water (100 ml) using an Accumet 950 pH/ion Meter (Fisher Scientific Co., Pittsburgh, PA). Chemical analyses were determined according to the Standard Methods of Analysis, Section 39, as described for the determination of meat and meat products (AOAC, 1990; AOAC, 1993)

### Listeriai Culture Preparation and Enumeration

*L. monocytogenes* Scott A was supplied by the National Animal Disease Center, Ames, IA (Dr. I. V. Wesley). Enumeration and identification of listeriae were followed from methods described by Lovette (1990), Benedict (1990), and Buchanan (1990). The bacteria were grown in Trypticase Soy Broth (TSB, Beckton Dickinson, Cockeysville, MD, BBL) supplemented with 0.6% yeast extract (YE, BBL). Listeriae were harvested after an incubation time of 18-20 h at 37°C representing the early stationary phase. Preliminary study showed that listerial cells reached the stationary phase at 16 h. Dilutions were made so that 0.1 ml suspension would yield  $10^7$ - $10^8$  cells/g of meat upon inoculation.

Recovery of *L. monocytogenes* Scott A after treatments was performed using Modified Oxoid Agar (MOX, Oxoid, Unipath Ltd., Hampshire, England) supplemented with Listeria Selective Supplement (SR 140, Oxoid) and Trypticase Soy Agar (TSA, BBL) supplemented with 0.6% yeast extract (BBL). Supplemented MOX supports healthy cells while TSA supports both healthy and injured cells. Cell survivors were enumerated after incubation at 32°C for 5 days. Plates were counted with the aid of a Darkfield Quebec Colony Counter (American Optical Co., New York, NY). Percent injury was calculated from the following equation:

$$\text{Percent injury} = (\text{Injured cells}/\text{Total cells}) \times 100 \%$$

Presumptive characteristics of *L. monocytogenes* Scott A were performed on Gram-reactions, catalase production, colony morphology, and motility on wet mount using a phase contrast microscope. Cultures of *L. monocytogenes* were Gram-positive,

and catalase-positive. On MOX, listerial colonies were black, round, low convex, and approximately 0.2-0.5 mm in diameter. On TSA, colonies were round, low convex, smooth surface, entire margin, dew drop-like in appearance, translucent, and similar in size to colonies on MOX. When exposed TSA agar plate to incident transmitted white light, colonies appeared bluish which looked like crushed glass under a low-power microscope. Young cultures of *L. monocytogenes* Scott A, incubated at 25°C, were used to detect the characteristic tumbling motility (Lovett, 1990; Bebedict, 1990; Buchanan; 1990).

### **Statistical Analysis**

A factorial design of 2x2x4 was used followed by a split-plot. Main effects were packaging (air and vacuum), temperatures (2°C and 48°C), and irradiation (0, 0.5, 1.0, 1.5 kGy) for both inoculated and un-inoculated samples. One pork patty was assigned to a combined treatment, with 16x2 treatments in total. At the end of the treatment, one sample was split into 2 portions and each was recovered on different growth media (MOX or TSA) in duplicate plating. Experiments were repeated four times on different batches of pork. Data were analyzed using the General Linear Model procedure (Statistical Analysis System, SAS, 1986). Linear regression equations were computed and D-values obtained from the reciprocal of the slopes.

## RESULTS AND DISCUSSION

Proximate analysis of fresh ground pork used in the experiments showed the composition of pork ground from lean tissues. Mean percent composition and standard deviation of protein, fat, and moisture were found to be  $18.1 \pm 0.2$ ,  $13.0 \pm 0.2$ , and  $67.9 \pm 0.1$  respectively.

Because non-sterilized samples were used in this study, un-inoculated control samples were treated in a similar manner as the inoculated samples. Un-inoculated controls were included to study the interference that background microflora might have on *L. monocytogenes* Scott A. Populations of aerobic, mesophilic microorganisms of un-inoculated ground pork ranged from log 2.4 to log 4.5 CFU/g with a mean initial count of log 3.6 CFU/g (data not shown). This level of initial contamination is negligible when compared to the inoculum of *L. monocytogenes* Scott A of log 7 to log 8 CFU/g in meat. Presumptive growth on supplemented Modified Oxoid Agar did not reveal typical listerial colony in any un-inoculated samples. Recovery of microorganisms from the irradiated, un-inoculated controls did not show any unusual radiation-resistant strains (data not shown). These results suggested that none of the microflora in un-inoculated samples would interfere with detecting colonies of *L. monocytogenes* Scott A that developed from the inoculated-pack study. Therefore, only listerial counts were used without any adjustment for the background microflora. Irradiation dosage, temperature, and packaging did not affect the pH of ground meat (Table 1).

When relatively mild heat and/or low doses of irradiation were applied, injury as well as lethality of cells may be found. Listerial growth of healthy cells plus injured cells on TSA and the growth of only healthy cells on MOX, as affected by irradiation, are presented in Figures 2 and 3. MOX yielded significantly ( $p < 0.001$ ) lower counts than TSA indicating the occurrence of cell injury which was calculated to be 3.7%. Both healthy and injured cells were highly significantly ( $p < 0.001$ ) reduced as irradiation dosage increased indicating their dose-dependent characteristic.

In this study, samples were placed in a heated water bath in a microwave oven to minimize the non-uniformity associated with microwave heating. Though different mechanisms had been proposed for microwave heating (Khalil and Villota, 1988; Dreyfuss and Chipley, 1980), several other researchers reported that microwaves inactivated microorganisms in a similar manner as in thermal processing (Heddleson and Doores, 1994; Vela and Wu, 1979; Lechowich et al., 1969; Goldblith and Wang, 1967). Maximum growth temperature for listeria was reported to be 45°C (Judge et al., 1989). Temperature increase above this point would result in listerial inactivation.

Heat treatments in this experiment, though were relatively mild, highly significantly ( $p < 0.001$ ) reduced the listerial numbers from log 5.8 to log 5.2 CFU/g (standard error of the means = 0.1). Kim (1992) found that approximately  $9 \times 10^7$  CFU/g (12%) of *L. monocytogenes* Scott A remained from the initial inoculum of approximately  $7 \times 10^8$  CFU/g following heating in a water bath (48°C, 2 h) in

Trypticase Soy Broth Yeast Extract medium. From our heating/cooling curve, the come up time and cool down time were adjusted and the heating time at a constant temperature of 48°C was evaluated to be 3 min. Twenty one percent healthy cells survived this heat treatment. It is not numerically valid to compare results from these two experiments as the heating time at 48°C from their experiment was not available for comparison. At the time of recovery, our data showed a significant ( $p < 0.05$ ) amount of injured cells, caused by heat treatment but not ( $p > 0.05$ ) by irradiation. This result implies that radiation injured cells, if any, had recovered by the time of recovery (<10 h after treatments), while heat injured cells had not.

Mackey and Derrick (1982) demonstrated that, following equivalent lethal treatments, *Salmonella typhimurium* required 9 h to repair heat (52°C, 30 min) injured cells and less than 2 h to repair gamma radiation (dose not included) injured cells. Our results suggested differences in repair time following exposure to different level of heating and irradiation.

Irradiation at 48°C resulted in consistently lower survivors than irradiation at 2°C (Figures 2 and 3). No significant ( $p > 0.05$ ) interaction effect between heating and irradiation was found. However, an additive effect of the two might be responsible for the lower counts observed when irradiation was performed at 48°C.

The lack of interaction between heating and irradiation in this study should not exclude their interaction effects in other combinations, or to other pathogens. For an interaction effect to occur, it is possible that each physical parameter needs to be applied at an energy level that maximize cell injury. Each parameter should

cause lethality on the microorganism being studied. In practice, where conditions in foods vary greatly, synergistic effects may not be obtained in all occasions.

However, the benefits of using several mild treatments to produce a combined effect should not be under-estimated.

Grecz et al. (1971) demonstrated that D-values of *Clostridium botulinum* 33A spores in ground beef decreased as irradiation temperatures (from -196°C to +95°C) increased. A sharp drop of radiation D-value was noted at approximately +80°C. It is evident from this study that physical parameters must reach a lethal point at the time interaction effects occur. Emborg (1974), however, did not find differences in radiation resistance of *Bacillus subtilis* when irradiation was carried out at 80°C and 100°C.

Recovery of *L. monocytogenes* Scott A were significantly ( $p < 0.001$ ) higher when irradiation and recovery procedures were carried out anaerobically versus aerobically. D-values of *L. monocytogenes* Scott A in oxygen-permeable and vacuum packages after exposure to irradiation at 2°C and at 48 °C are presented in Table 2. D-values found under anaerobic conditions were higher than D-values in air. This implies that, at the same level of irradiation or heat treatment, higher number of *L. monocytogenes* was recovered under anaerobic conditions. Knabel et al. (1990) also recovered higher numbers of heat-injured cells of *L. monocytogenes* in strictly anaerobic conditions compared to aerobic conditions. They suggested that, after heating, catalase and superoxide dismutase are inactivated preventing the cells from growing in the presence of toxic oxygen radicals found in aerobic

conditions.

### CONCLUSIONS

1. Irradiation at 48°C resulted in greater destruction of *L. monocytogenes* Scott A than irradiation at 2°C, particularly within the dose range studied. However, the rate of destruction, as indicated by D-values, appeared to be higher at 2°C than at 48°C.
2. Vacuum packaging, during irradiation and recovery, resulted in significantly ( $p < 0.001$ ) higher survivors of *L. monocytogenes* Scott A as opposed to air packaging.
3. From this study, the most effective treatment to control *L. monocytogenes* Scott A in ground pork was irradiation at 2°C, in air.

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Table 1. Mean pH measurement in ground pork at different irradiation doses

Treatment	Dose (kGy)			
	0	0.5	1.0	1.5
Refrigerated/Air	5.94	5.92	5.96	5.93
Refrigerated/Vacuum	5.97	5.96	5.93	5.96
Heated/Air	5.99	5.98	5.98	5.97
Heated/Vacuum	6.01	6.00	5.99	5.98

Table 2. Radiation D-values of healthy and injured-cells of *L. monocytogenes* Scott A

Treatment	Regression equation	R-square	D-value (kGy)
<b>Healthy and injured cells</b>			
Refrigerated/air	$Y = 8.15 - 3.19x$	0.96	0.31
Refrigerated/vacuum	$Y = 8.15 - 2.69x$	0.98	0.37
Heated/air	$Y = 6.95 - 2.82x$	0.99	0.35
Heated/vacuum	$Y = 7.58 - 2.37x$	1.00	0.42
<b>Injured cells</b>			
Refrigerated/air	$Y = 8.06 - 3.16x$	0.97	0.32
Refrigerated/vacuum	$Y = 7.96 - 2.57x$	0.97	0.39
Heated/air	$Y = 6.84 - 2.91x$	1.00	0.34
Heated/vacuum	$Y = 7.20 - 2.38x$	0.98	0.42

Least significant difference ( $p=0.05$ ) for differences among slopes = 0.83

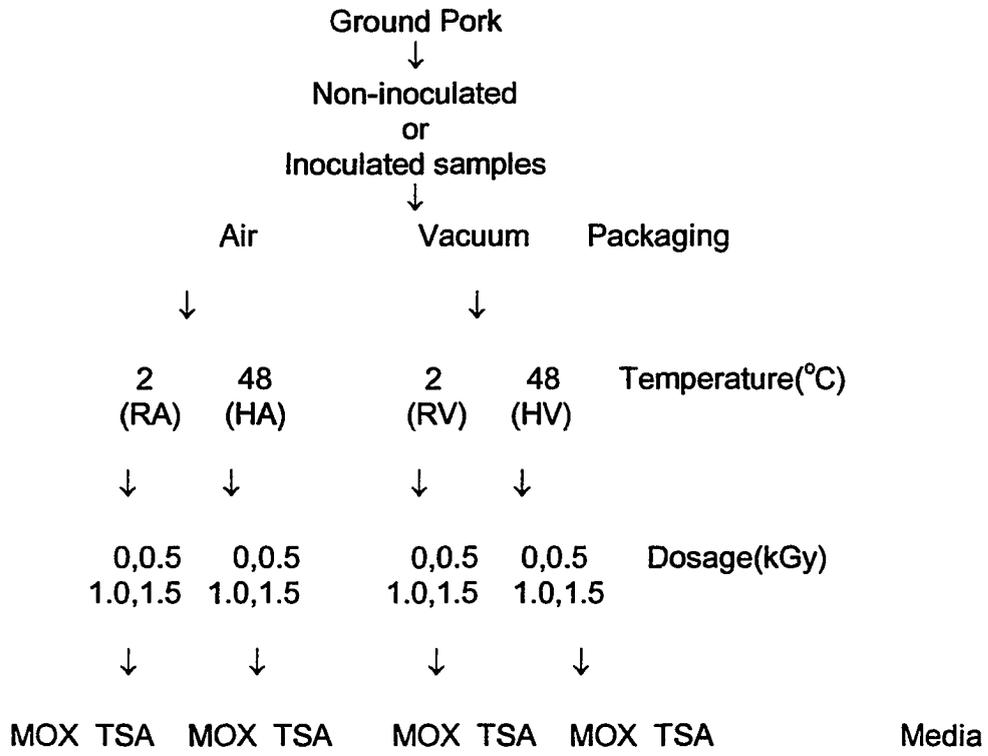


Figure 1. Experimental design for the combined treatments on ground pork un-inoculated and inoculated with *L. monocytogenes* Scott A

RA, RV = Refrigerated/air and refrigerated/vacuum

HA, HV = Heated/air and heated/vacuum

MOX = Modified Oxoid Agar    TSA = Trypticase Soy Agar

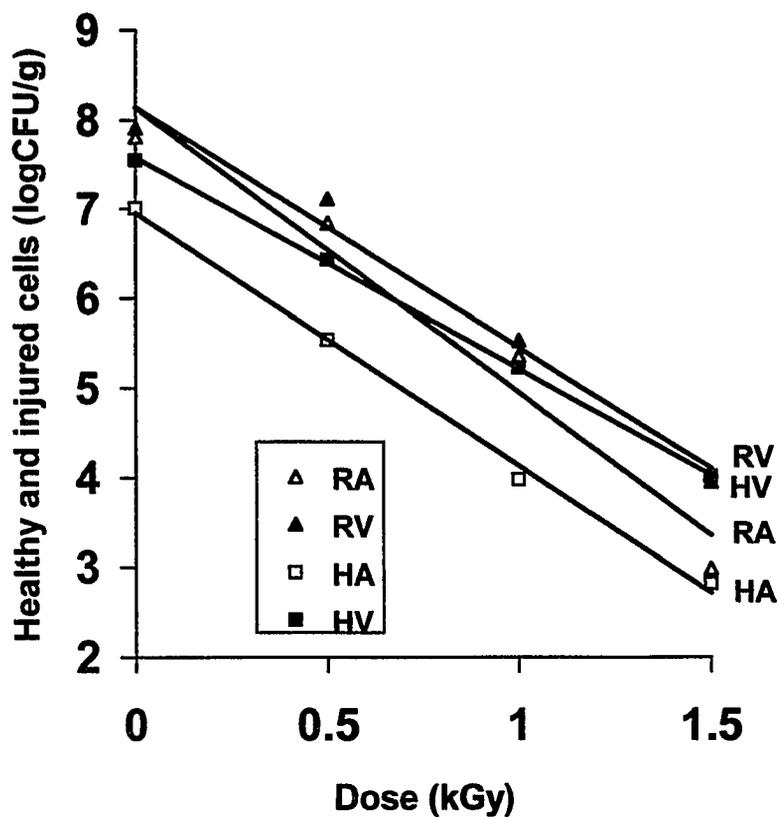


Figure 2. Effect of irradiation temperature on healthy and injured cells of *L. monocytogenes* Scott A in ground pork packaged in air and vacuum. RA, RV = refrigerated/air and refrigerated/vacuum; HA, HV = heated/air and heated/vacuum, standard error of the means = 0.42

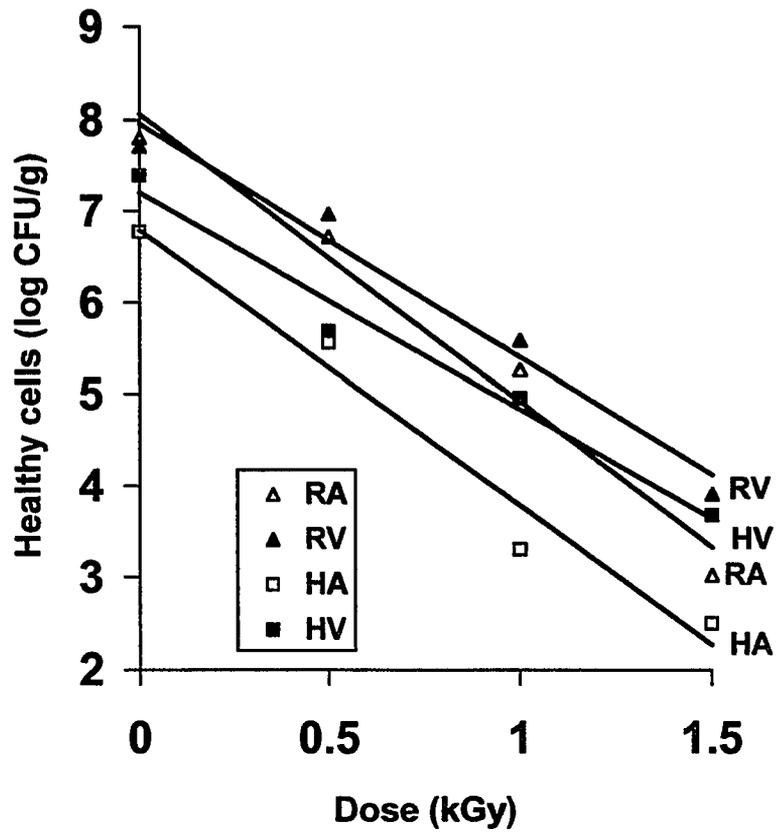


Figure 3. Effect of irradiation temperature on healthy cells of *L. monocytogenes* Scott A in ground pork packaged in air and vacuum. RA, RV = refrigerated/air and refrigerated/vacuum; HA, HV = heated/air and heated/vacuum, standard error of the means = 0.42

**STORAGE LIFE EXTENSION OF GROUND PORK AS AFFECTED BY HEATING  
AND RADURIZATION**

A paper to be submitted to the Journal of Food Protection

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**ABSTRACT**

Fresh ground pork patties, either packaged in air or under vacuum, were subjected to irradiation (1 kGy) at the irradiation temperatures of 2°C and 48°C. Mesophiles, psychrotrophs, and lactic acid bacteria were enumerated weekly, until spoilage, to determine the storage-life of ground pork upon extended storage at 2°C.

Irradiation at 1 kGy significantly ( $p < 0.001$ ) reduced the mesophiles and psychrotrophs in ground pork by 1.5 log cycles at week 0. Lactic acid bacteria were less affected ( $p < 0.001$ ) showing a 1.2 log reduction. Non-irradiated samples,

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packaged in air and treated at the temperatures of 2°C or 48°C, reached the spoilage level at 5 and 6 days of storage respectively. Non-irradiated, vacuum packaged samples spoiled after approximately 16 (samples treated at 2°C) to 26 days (samples treated at 48°C). Ground pork, irradiated at 2°C, showed a shelf-life of 23 days when packaged in air and 26 days under vacuum. When irradiation was performed at 48°C, the shelf-life of ground pork in aerobic and vacuum packaging was 31 days and 35 days respectively.

## INTRODUCTION

Fresh meat surfaces may be contaminated with a wide variety of micro-organisms. At room temperature, coliforms, micrococci, bacilli and clostridia may be present (Jay, 1992). At low temperature, psychrotrophic bacteria such as *Pseudomonas* spp. predominate followed by *Moraxella-Acinetobacter* spp. (Davidson et al., 1973) and *Brochothrix thermosphacta* (Gardner, 1981).

Vacuum packaging extends the shelf-life of meat by minimizing lipid autoxidation and limiting the access of oxygen to aerobic spoilage microorganisms. Exclusion of oxygen shifts the normally present microflora from aerobic, Gram-negative *Pseudomonas* spp. to the facultative, Gram-positive lactobacilli (Henry et al., 1983) which results in a slower development of spoilage. Gamma irradiation at a dose of 1 kGy and vacuum packaging has been shown to extend the shelf-life of pork loins (Mattison et al., 1986) and ground pork (Ehioba, 1987; Ehioba et al., 1987). Little is known about the microbiological safety of vacuum packaged ground

pork irradiated with an electron accelerator at a higher temperature.

Irradiation at higher temperatures has been shown to result in lower resistance of bacteria as compared to irradiation at refrigeration temperatures (El-Bisi et al., 1966). The direct effect of radiation is physical in nature and is temperature independent. The secondary effect of irradiation, however, is a chemical reaction and thus increases as temperature increases. The objective of this study was to apply the hurdle concept by using several mild treatments, namely; heating at 48°C, 1 kGy irradiation, exclusion of oxygen, and storage at refrigeration temperature, to control microbial spoilage. The temperature chosen was mild, below 50°C, to secure meat in an uncooked stage. A dose of 1 kGy, which has been approved by the Food and Drug Administration (FDA) to render "trichina-safe" pork and to prolong storage-life, was used (FDA, 1985).

## **MATERIALS AND METHODS**

### **Preparation of Pork**

Fresh pork was obtained from a meat packer in Iowa and prepared for treatments in the Meat Laboratory, Iowa State University. Meat was ground twice through 0.6 cm followed by 0.3 cm pore size grinder plates (The Biro Manufacturing Company, Marble Head, OH). Approximately 100 g of ground pork were formed into patties of 2 cm thick by 10 cm diameter by hand with the aid of a molder ring. Samples were packed in air or under vacuum (Ruhestellung, Multivac, Sepp Haggemullur KG, West Germany) at 1 kPa. Polyethylene packages and vacuum

packages with oxygen barrier property of 0.46-0.62 cm<sup>3</sup>/1m<sup>2</sup>/atm/20°C at 0%RH/24h represented oxygen permeable and oxygen impermeable packages respectively. Samples were subjected to different combination treatments of heating and/or irradiation (Figure 1). After treatments, pork patties were stored at 2°C for 0, 1, 2, 3, and 4 weeks for a microbiological analysis.

### **Treatment by Temperature**

Refrigerated samples were held in cold storage overnight to ensure the internal temperature of 2°C. Samples subjected to heat treatment were heated in a thermal processing unit (Raucherzeuger Goliath II, Maurer and Sohne, Rauch-und-Warmetechnik Gmbh and Co., KG, West Germany) to reach the final temperature of 48°C. A number of extra patties was treated in a similar manner as controls. The temperature change of the pork was measured by a Thermistor Digital Temperature Tester to ± 0.2% (Hantover, Kansas City, MO) with a heavy duty meat probe. During heating, temperature of the thermal processing unit as well as temperature of the pork were recorded. Heating and cooling curves were constructed and the heating time evaluated based on a mathematical method (Stumbo, 1965). Samples designated as non-irradiation, heated/air and heated/vacuum (HA/N or HV/N) were transferred to cold storage (2°C) as soon as the desired temperature was reached. Samples prepared for irradiation were placed in insulated card-board boxes for irradiation.

**Irradiation**

Ground pork was irradiated at the Linear Accelerator Facility of the Meat Laboratory, Iowa State University, at refrigeration and at high temperature in insulated card-board boxes. Temperature changes of ground pork before and after irradiation were recorded. During irradiation, the temperature of refrigerated samples was estimated to be between 4 to 5°C, and those of the heated samples were 45 to 46°C. A dose of 1.0 kGy was achieved by adjusting the power level (1 or 3 kW) of the Linac and the conveyor speed (m/min). Absorbed doses were measured by placing 99% pure alanine pellets (Bruker Instruments, Inc., Billerica, MA), on the upper and the lower surfaces of the pre-packaged patties and the average dosage used. An Electron Paramagnetic Resonance instrument (EMS 104, Bruker Analytische Messtechnik, Karlsruhe, Germany) was used to determine the free radicals formed and thus the absorbed doses.

**Proximate Analysis and pH Measurements**

Protein analysis was performed by using the Combustion Analyzer Instrument (Leco FP 428, Leco Corp., St. Joseph, MI) standardized with ethylene diamine tetra acetic acid (EDTA, Leco Corp.). Percent crude protein was estimated from nitrogen content (per cent) x 6.25. Fat content was extracted from 5 g samples using n-hexane extraction in Soxhlet apparatus. Moisture content was analyzed by drying ground pork samples in the Gravity and Mechanical Convection Constant Temperature Cabinets (GCA Precision Scientific Model 27, Gravity Convection Ovens, GCA Corp., Chicago, IL). The weight difference, before and after drying,

represented the moisture content of the meat . The pH was measured from the filtered slurry of ground pork (10 g) diluted with distilled water (100 ml) using an Accumet 950 pH/ion Meter (Fisher Scientific Co.). Chemical analyses were determined according to the standard methods of analysis, Section 39, as described for the determination of meat and meat products (AOAC, 1990; AOAC, 1993).

### **Microbiological Evaluation**

Ground pork samples were transferred aseptically to pre-filtered Stomacher bags (Prepit, Integrated Bio-Solutions, Inc.). One hundred ml of 0.1% peptone water (Difco Laboratories, Detroit, MI) was added to the samples and the mixture blended in a Stomacher (Tekmar Co., Cincinnati, OH) for 2 min. The supernatant was used to make appropriate dilutions and spread onto microbiological media.

Samples in oxygen permeable packages were evaluated aerobically. Mesophilic counts were performed using Trypticase Soy Agar (TSA, Beckton Dickinson, BBL, Cockeysville, MD) supplemented with 0.6% yeast extract (YE, BBL) as a growth medium and incubated at 37°C for 5 days. Psychrotrophic counts were performed likewise using the same medium and incubated at 10°C. Lactic acid bacteria were enumerated on De Man Rogosa Sharp agar (MRS, BBL) and incubated at 37°C. Plates were counted with the aid of a Darkfield Quebec Colony Counter (American Optical Co., New York, NY).

Vacuum packaged samples were evaluated for mesophilic, psychrotrophic, and lactic acid bacteria in a parallel manner in anaerobic condition. Mesophilic and psychrotrophic counts were made on TSAYE (BBL) agar plates plus 1% soluble

starch (Fisher Scientific, Fair Lawn, NJ), and lactic acid bacteria on MRS (BBL) agar. Anaerobic atmosphere was generated in the anaerobic jars with GasPak 150 pouches including indicators (BBL) at the same temperature and time as previously described. Bacteria were enumerated, in duplicates, immediately after treatments while holding the temperature of the patties between 2°C to 4°C.

Microbiological spoilage levels of  $10^7$  mesophilic or psychrotrophic colony forming units (CFU/g) (Kraft and Ayers, 1952) or  $10^8$  lactobacilli (Hanna et al., 1979) were defined as a spoilage level. Samples were discontinued when either indicator of spoilage was reached.

Since the bacterial count was made weekly, samples might have reached pre-defined spoilage levels between two consecutive weeks. When the spoilage level was reached before the next bacterial count was made, the “expected” storage-life was derived from the bacterial growth curves. Experiments were performed in triplicates except for the storage time of 5 weeks which it was performed only once.

### **Statistical Analysis**

A factorial design of 2x2x2 was used. Main effects were packaging (air and vacuum), temperature (2°C and 48°C), and irradiation (0 and 1.0 kGy). One pork patty was designated for each treatment and experiments were repeated three times on different batches of pork. Data were analyzed using the General Linear Model procedure (Statistical Analysis System, SAS, 1986).

## RESULTS AND DISCUSSION

Proximate analysis of fresh ground pork was found to be  $22.3 \pm 0.9$ ,  $10.4 \pm 0.6$ , and  $68.9 \pm 0.3$  percent for means and standard deviation of protein, fat, and water respectively. Mesophilic, psychrotrophic, and lactic acid bacterial counts of fresh ground pork are presented in Figures 2, 3, and 4. Initial mean contamination of these bacteria were relatively low as would be expected in a controlled environment such as the Meat Laboratory. Ehioba (1987) found similar counts on mesophiles (log 4.0 CFU/g) and psychrotrophs (log 4.2 CFU/g) but lower lactic acid bacterial counts (log 1.0 CFU/g) in their fresh ground pork processed from the same Meat Laboratory. Slightly lower levels of initial contamination were reported as aerobic plate counts of log 3.3 CFU/g (O'Connor et al., 1993) and approximately log 3.2 CFU/g (Buchanan et al., 1992).

Irradiation at 1 kGy highly significantly ( $p < 0.001$ ) reduced mesophilic, psychrotrophic, and lactic acid bacterial numbers at week 0 (Figures 2, 3 and 4). Mesophiles and psychrotrophs showed similar sensitivity to radiation and both were more sensitive than lactic acid bacteria. Irradiation at 1kGy reduced their numbers by log 1.5, 1.5, and 1.2 respectively. Lebepe et al. (1990) and Ehioba et al. (1987) also reported lactic acid bacteria to be least affected by irradiation, at 3 and 1 kGy respectively. Lactic acid bacteria have been shown to be relatively resistant to radiation by several other authors (Schweigert et al., 1954; Hasting et al., 1986;

Patterson and Grant, 1990). Counts of all three spoilage bacteria continued to be significantly ( $p < 0.001$ ) lower in irradiated samples throughout the storage periods.

A heating and cooling curve in a thermal processing unit was plotted and the process time at 48°C was evaluated using a mathematical method (Stumbo, 1965) to be 34.8 min. Heating at 48°C/34.8 min is considered mild and contributes to cell stress in bacteria which would probably recover. Heating significantly ( $p < 0.05$ ) reduced mesophiles and lactic acid bacteria by 74% and 76%. Psychrotrophic counts, though not significantly ( $p = 0.08$ ) lower in the heated samples, were reduced in number by 77%. The insignificant effect of heating on psychrotrophs was due to the large variations of their means, in spite of the highest log reduction among these three types of bacteria. Sensitivity to heating, as indicated by log reduction of survivors, may be presented in the order of mesophiles < lactic acid bacteria < psychrotrophs. After one week of storage at 2°C, all three types of microflora recovered from heating and the effects of temperature was reversed.

Packaging had no significant ( $p > 0.05$ ) effects on the growth of mesophiles, psychrotrophs, and lactic acid bacteria at week 0 (Figures 2, 3, and 4). After week 1, however, survivors from heat and/or irradiation of mesophiles and psychrotrophs in vacuum packaging were significantly ( $p < 0.01$ ) lower than those in air. The effect of vacuum packaging to control aerobic mesophilic/psychrotrophic bacteria was extended throughout the storage time. Lactic acid bacterial counts were not significantly ( $p > 0.05$ ) different in vacuum packaging or in air, at any storage time. This may be explained by the oxidation-reduction potential ( $E_h$ ) in the packages and

in the requirement for nutrients by injured cells. Following heating and/or irradiation, a certain portion of bacterial populations is injured. Optimum conditions of recovery are required to reverse the injury. Aerobic mesophiles/psychrotrophs require oxygen to proliferate while lactic acid bacteria are microaerophilic in nature, and require only a small amount of oxygen to multiply (Jay, 1992). As a result, mesophilic and psychrotrophic counts were lower in vacuum packaging while lactic counts were not affected by exclusion of air. In vacuum packaging, the growth of lactic acid bacteria is favored because of the increased level of CO<sub>2</sub> and a lower E<sub>h</sub> (Seideman and Durland, 1983). In the present study, lactic acid bacteria grew slowly in both air and vacuum packaged meat. Kraft and Ayers (1952) and Hanna et al. (1979) defined microbial spoilage to be log 7.00 CFU/cm<sup>2</sup> mesophiles or psychrotrophs and log 8.00 CFU/cm<sup>2</sup> lactobacilli. In this study, lactic acid bacterial counts never reached the pre-defined spoilage level throughout the period of storage. All samples were spoiled according to mesophilic and/or psychrotrophic counts before the pre-defined level of log 8.00 CFU/g lactobacilli was obtained.

Mean pH values of pork, of the combined treatments, are shown in (Table 1). The initial mean pH of pork in this study (5.9 to 6.0) was slightly higher than would be expected in normal pork of pH 5.6 to 5.7 (Judge et al., 1989) but lower than the dark-firm-dry pork of pH above 6.0 (Judge et al., 1989). At week 0, the pH of heated samples was slightly, but not significantly ( $p > 0.05$ ), higher than their refrigerated counterparts. Jay (1992) stated that cooked or partially cooked meats have higher pH than uncooked meats.

In this study, treatments had no significant ( $p > 0.05$ ) effects on pH but storage time ( $p < 0.001$ ) and treatment-time interaction ( $p < 0.01$ ) did. The onset of spoilage is accompanied by a rise in pH (Turner, 1960; Pearson, 1968; Jay, 1992) particularly in oxygen-permeable packaged meats. In this study, pH values of non-irradiated ground pork, packed in air, progressively increased upon extended storage. On the contrary, pH values of vacuum packaged pork samples, as well as those of irradiated samples, followed different patterns. Irradiated pork, regardless of packaging type, showed a small increase (0.11 to 0.14 units) in pH values after week 2, then declined. The ultimate pH, however, was never as low as in the fresh pork at week 0. PH values of non-irradiated, vacuum packaged pork either remained almost unchanged (refrigerated samples) or progressively increased (heated samples). Sutherland et al. (1975) did not observe the trend of pH increase in vacuum packaged beef. Jaye et al. (1962) also observed that the pH of ground beef, packed in an oxygen impermeable film, tended to fall or remain constant rather than rise. Lebepe (1989) did not find significant differences in pH between irradiated (3 kGy) and non-irradiated pork loins, vacuum packed, during the 41 days of storage at 2 to 4°C. When red meats are stored at 0 to 5°C under packaging conditions that restrict the entry of O<sub>2</sub> or the egress of CO<sub>2</sub>, the ultimate flora is dominated by the lactic acid bacteria (Jay, 1992). The lactics were relatively resistant to irradiation and remained viable at a dose of 1 kGy (Ehioba et al., 1987). These lactic acid bacteria, which are comprised of a large group of hetero- and

homo-fermentative bacteria, may be responsible for the variable pH values observed during prolonged storage.

Based on the pre-defined spoilage definition, the first sample to spoil was the non-irradiated, refrigerated, aerobic packaged pork (RA/N). Shelf-life of ground pork, from all combined treatments, is presented in Table 2. Aerobic mesophiles and psychrotrophs of RA/N treatment reached the pre-defined level on day 5 (Figures 2 and 3). Next, were the non-irradiated, heated, aerobic packaged (HA/N) samples which reached the same definition of spoilage on day 6. The two last samples to spoil were ground pork patties, irradiated to 1 kGy at the irradiation temperature of 48°C, either packaged in air or under vacuum (HA/I and HV/I). When non-irradiated and irradiated samples were compared, the benefits of irradiation as indicated by the extra storage-life it brought to the combined treatments may be ranked in the order of heated/air(HA) > refrigerated/air (RA) > heated/vacuum (HV) > refrigerated/vacuum (RV). Samples packed under vacuum gained less benefit (9 and 10 days) from 1 kGy electron irradiation as compared to samples packed in air (18 and 25 days). This implies that irradiation and recovery under vacuum, at low or high temperature, is not as effective as irradiation and recovery in air for the spoilage microbial control. Irradiation followed by aerobic recovery, particularly at a higher temperature, exhibits maximum bactericidal effects. Storage-life of combination treatments of irradiated samples, packaged in air, were extended for over two weeks over their non-irradiated counterparts.

Although 1 kGy irradiation yielded maximum benefits to the heated/air (HA/I) samples, their storage-life appeared to be shorter than the storage-life of heated/vacuum (HV/I). At the end of week 4; counts of mesophiles, psychrotrophs, and lactic acid bacteria in the samples, irradiated at 48°C, were similar. The counts were in the order of log 6.00 CFU/g for all three kinds of bacteria. It might be predicted that if HA/I and HV/I samples were to spoil at their previous rates, their storage-life would be longer than 4 weeks. From the 3 replications of experiment, ground pork (HV/I treated) from only one replication had reached the pre-defined spoilage level on week 5. Ground pork from the other 2 replications did not reach the spoilage level even after being stored for 5 (in one replication) and 6 weeks (in the other replication). Difference in storage life may be expected from the diversity of microorganisms in different batches of meat. It is clear, however, that HV/I treated samples from this study showed a storage life of a minimum of 5 weeks. Vacuum packaging is known to minimize oxidation of meat lipids (Ranken, 1989; Lebepe et al., 1990) and shifts the microflora from predominantly Gram-negative bacteria to slower growing lactobacilli (Hanna et al., 1977; Henry et al., 1983; Seideman et al., 1976; Vanderzant et al., 1982; Jay, 1992). It is, therefore, justified to conclude that HV/I treatment, i.e. vacuum-packaged and irradiated at 48°C, was the most beneficial combination to control microbial spoilage. It has been shown that exclusion of oxygen minimizes the oxidative rancidity initiated by irradiation in beef (Mead, 1954). Therefore, vacuum packaging may be used to preserve the fresh meat aroma and flavor caused by oxidation.

There is no available data to compare the storage-life of ground pork irradiated at an elevated temperature. Ehioba et al. (1987) reported the storage-life of vacuum-packaged ground pork, irradiated (1 kGy) at ambient temperature (18-21°C), to be 10-11 days at 5°C storage temperature. In this study, the storage-life of vacuum-packaged ground pork, irradiated at 2°C, appeared to be 26 days. The longer storage-life might have resulted from the lower storage temperature in this study (2°C) and the temperature control during irradiation (2°C).

### CONCLUSIONS

Mesophiles and psychrotrophs were found to be equally radiation sensitive while lactic acid bacteria were the most resistant. Non-irradiated samples, packaged in air, showed the storage life of approximately 5-6 days at 2°C. Their vacuum packaged counterparts showed a storage life of approximately 16-26 days. Irradiation, at 1kGy, added extra shelf-life of 18-25 days to air-packaged ground pork and 9-10 days to vacuum-packaged pork. The longest storage life of approximately 5 weeks was shown in ground pork packaged under vacuum, and irradiated at 48°C.

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Table 1. Mean pH values of non-irradiated and irradiated ground pork stored at 2°C

Treatment		Storage Time (weeks)				
		0	1	2	3	4
RA	N	5.90	6.01	6.23	N/A	N/A
	I	5.96	6.05	6.10	6.06	6.04
RV	N	5.97	6.01	5.99	N/A	N/A
	I	5.92	6.01	6.03	6.01	5.99
HA	N	6.00	6.08	6.13	N/A	N/A
	I	5.98	6.11	6.11	6.07	6.07
HV	N	6.02	6.03	6.10	6.14	N/A
	I	5.99	6.06	6.10	6.05	6.05

RA = Refrigerated/air packaging

RV = Refrigerated/vacuum packaging

HA = Heated/air packaging

HV = Heated/vacuum packaging

N = Non-irradiated samples

I = Irradiated samples

N/A= Non-applicable due to spoilage of samples

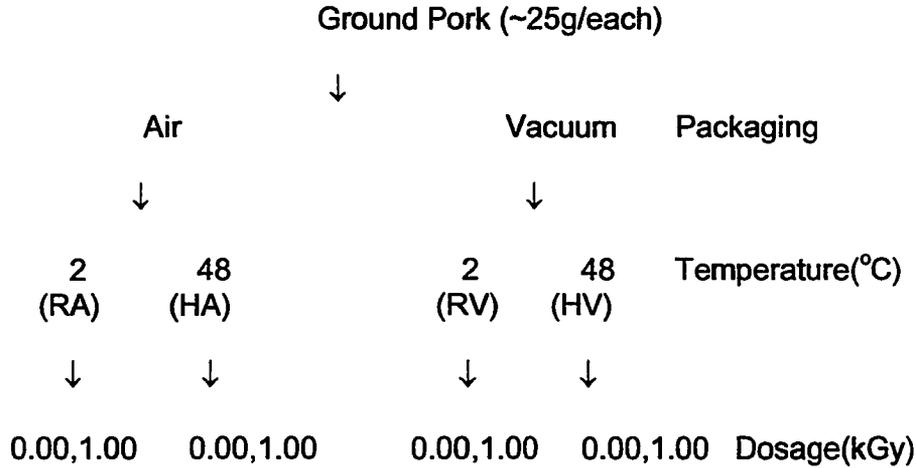
Standard error of the means = 0.03

Table 2. Storage-life extension in combined treatments, irradiated at 1kGy, and stored at 2°C

Treatment	Expected Storage-life (days)	Extra Storage-life (days)
<b>Non-Irradiated</b>		
Refrigerated/air packaging	5	-
Refrigerated/vacuum packaging	~16 <sup>1</sup>	-
Heated/air packaging	6	-
Heated/vacuum packaging	26 <sup>1</sup>	-
<b>Irradiation</b>		
Refrigerated/air packaging	23	18
Refrigerated/vacuum packaging	26	10
Heated/air packaging	~31 <sup>2</sup>	25 <sup>2</sup>
Heated/vacuum packaging	~35 <sup>2</sup>	9 <sup>2</sup>

<sup>1</sup> Days of storage-life derived from growth curves

<sup>2</sup> Days of storage-life obtained from 1 replication, while all other numbers were obtained from 3 replications



After treatments, samples were stored at 2°C for 0, 1, 2, 3, 4 weeks  
 Mesophiles, psychrotrophs, lactic acid bacteria were recovered weekly  
 Proximate analysis (week 0), pH measurement (weekly)

Figure 1. Experimental design for shelf-life extension study (RA and RV = refrigerated/air and refrigerated/vacuum packaging; HA and HV = heated/air and heated/vacuum packaging respectively)

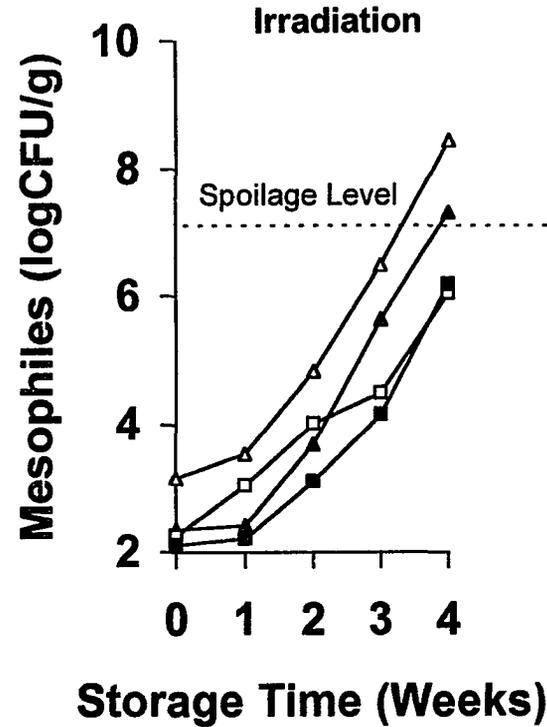
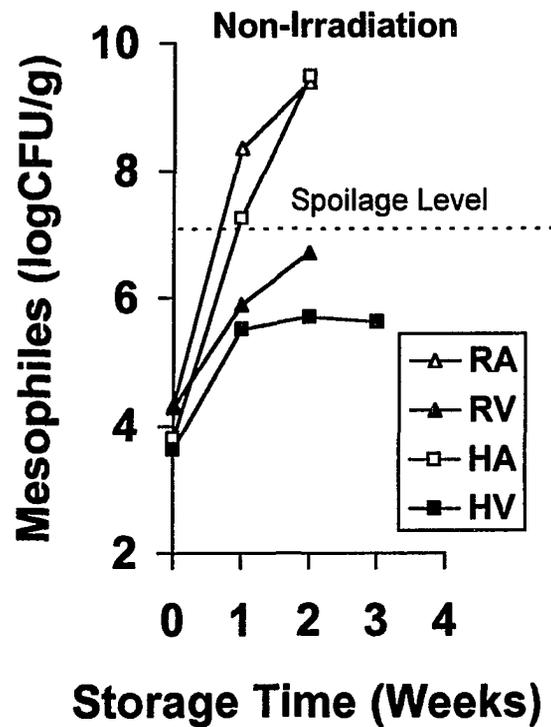


Figure 2. Effects of irradiation temperature on mesophilic bacteria in ground pork in vacuum and oxygen permeable packaging stored at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.31, 0.65, 0.60, 1.09 at week 0, 1, 2, and 3 respectively; SEM was not available on week 4)

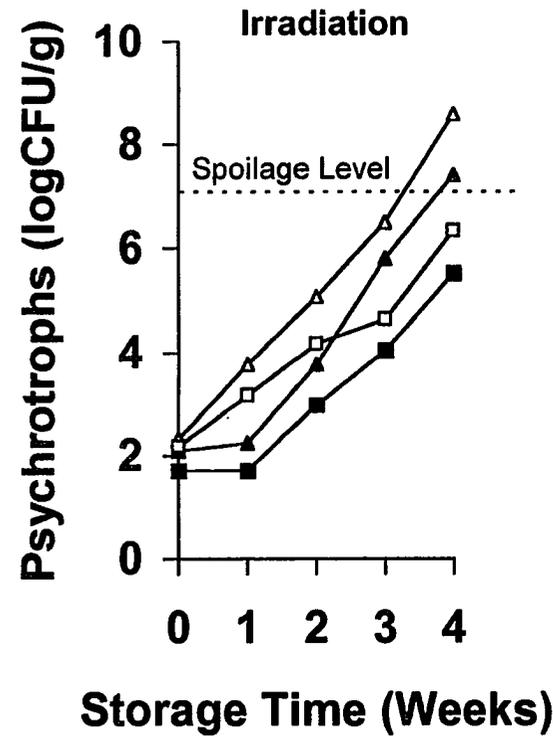
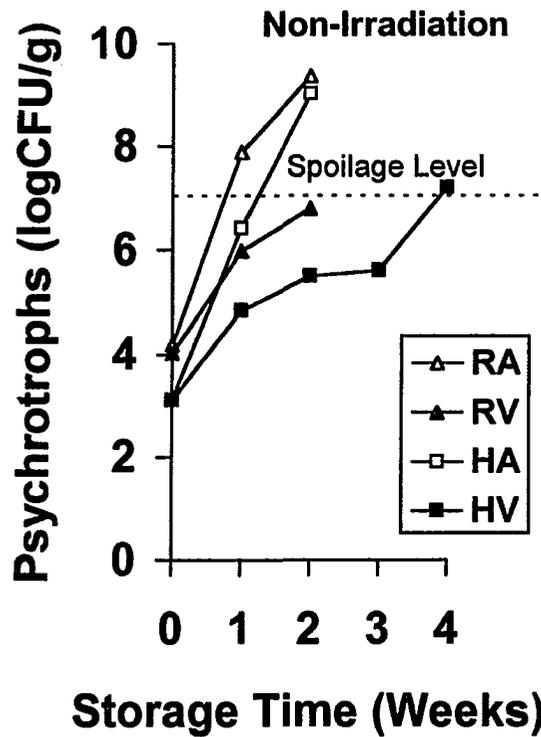


Figure 3. Effects of irradiation temperature on psychrotrophic bacteria in ground pork in vacuum and oxygen - permeable packaging stored at 2°C (RA=refrigeration, oxygen-permeable packaging, RV=refrigeration, vacuum packaging, HA=heat, oxygen-permeable packaging, HV=heat,vacuum packaging; SEM (standard error of the means) = 0.48, 0.73, 0.69, 1.11 at week 0, 1, 2, and 3 respectively; SEM was not available on week 4)

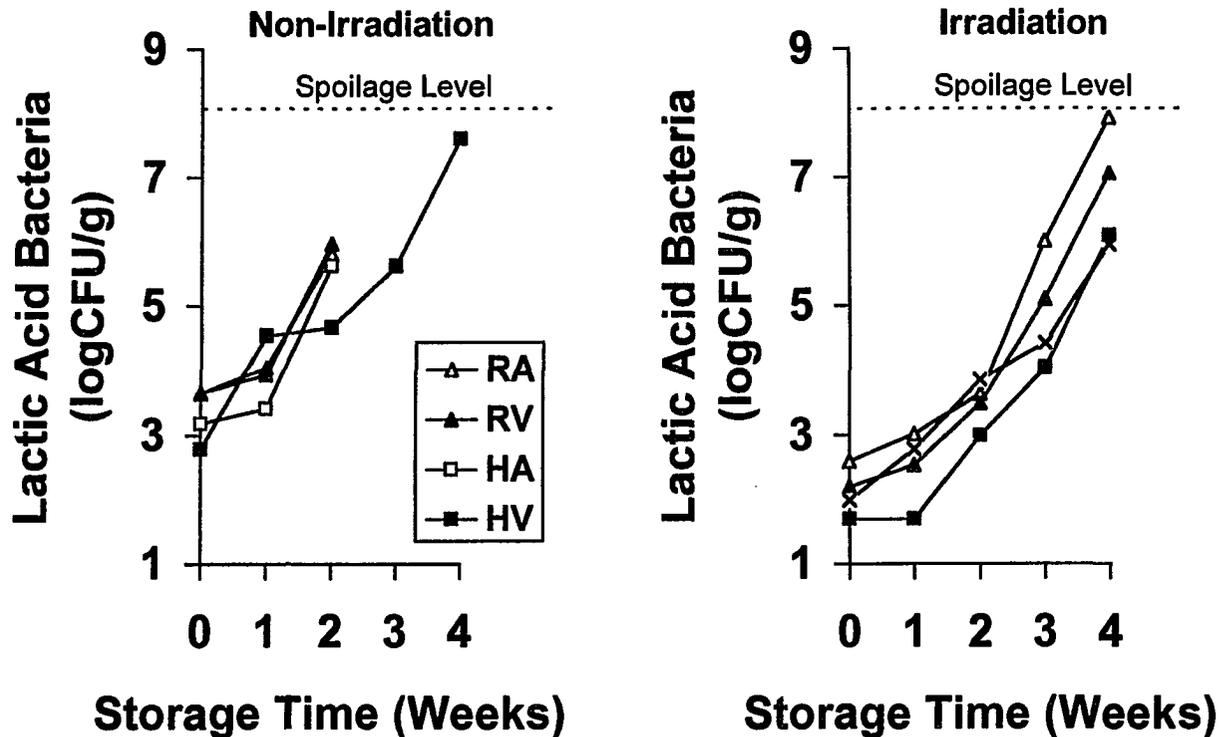


Figure 4. Effects of irradiation temperature on lactic acid bacteria in ground pork in vacuum and oxygen permeable packaging stored at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.36, 0.64, 0.54, 0.90 at week 0, 1, 2, and 3 respectively; SEM was not available on week 4)

**HEATING AND RADURIZATION EFFECTS ON THIOBARBITURIC ACID,  
HUNTER VALUES, AND SENSORY CHARACTERISTICS OF VACUUM- AND  
AEROBIC-PACKAGED GROUND PORK**

A paper to be submitted to the Journal of Food Science

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**ABSTRACT**

Ground pork patties, packaged in air and under vacuum, were subjected to irradiation (1 kGy) at the irradiation temperatures of 2°C and 48°C and stored at 2°C for 4 weeks. Color and odor, performed by untrained panelists, as well as Hunter L, a, and b values were evaluated on uncooked samples. Flavor, aroma, tenderness, juiciness and thiobarbituric acid numbers (TBA) were measured on broiled samples.

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At week 0, panelists significant ( $p < 0.01$ ) preferred the odor of non-irradiated to irradiated pork. After broiling and/or extended storage to week 1, there was no preference ( $p > 0.05$ ) in odor scores between irradiated and non-irradiated pork. At week 2, however, the odor of non-irradiated pork packaged in air, was less preferred ( $p \leq 0.001$ ) due to spoilage. TBA values of all irradiated pork were slightly higher than that of non-irradiated pork except from the aerobic-packaged, refrigerated (RA/I) samples. TBA values of RA/I increased uninterruptedly and exceeded 1.0 mg/1000g within 1 week. There was no correlation ( $p > 0.05$ ) between TBA values and odor scores. Vacuum-packaged pork, irradiated at 48°C, showed similar or higher sensory scores to its non-irradiated counterparts in all attributes, regardless of the extended storage time.

## INTRODUCTION

Ground pork is one of the most perishable of red meat products. Its storage life is generally less than a week in oxygen-permeable film at refrigeration temperatures. Atmospheric oxygen contributes to discoloration, lipid autoxidation and to the growth of aerobic microorganisms in meats. Butler et al. (1953) demonstrated that bacteria caused an increase in the rate of metmyoglobin formation leading to discoloration in prepackaged retail beef cuts. Several investigators have reported on the use of vacuum packaging to extend the shelf life of fresh meat beyond the traditional storage period of five days (Christopher et al., 1979; Sutherland et al., 1977).

The Food and Drug Administration (FDA) has approved the use of 0.3-1.0 kGy gamma radiation dose to render pork "trichina safe" and to prolong its shelf life (FDA, 1985). It appears that shelf life of fresh meats can be extended by radurization followed by refrigeration. The use of low doses of irradiation, combined with vacuum packaging, could prolong the shelf life of fresh pork beyond the use of vacuum packaging alone (Grau, 1983).

The hurdle or barrier concept is the application of several mild treatments, working in concert, to control the spoilage of foods while retaining their qualities. Each treatment, when applied alone, is not enough to achieve the desired effect of food preservation. The objective of this study was two fold: the first objective was to evaluate the effect of irradiation at two temperatures (2 and 48°C) on the qualities of ground pork as measured by physical-chemical methods and by sensory evaluation. The second objective was to find the best combination of treatments that would yield pork with acceptable qualities after storage at refrigeration (2°C) temperatures.

## **MATERIALS AND METHODS**

### **Preparation of Pork**

Fresh pork was obtained from a meat packer in Iowa and prepared for treatments in the Meat Laboratory, Iowa State University. Meat was ground twice through 0.6 cm followed by 0.3 cm diameter pore size grinder plates (The Biro Manufacturing Co., Marble Head, OH). Approximately 100 g of ground pork were formed into patties of 2.0 cm thick by 10.0 cm diameter by hand with the aid of a

molder ring. Samples were packed in air or under vacuum (Ruhestellung, Multivac, Sepp Haggenmuller KG, West Germany) at 1 kPa. Polyethylene packages and vacuum packages with oxygen barrier property of 0.46-0.62 cm<sup>3</sup>/m<sup>2</sup>/atm/20°C at 0%RH/24h represented oxygen permeable and oxygen impermeable packages respectively. Samples were subjected to different combination treatments of heating and/or irradiation (Figure 1). After treatments, samples were stored at 2°C for 0, 1, 2, 3, and 4 weeks for analysis.

#### **Treatment by Temperature**

The refrigerated samples were held in cold storage (2°C) overnight. Samples at high temperature were heated in a thermal processing unit (Raucherzeuger Goliath II, Maurer and Sohne, Rauch-und-Warmetechnik GmbH and Co. KG, West Germany) to reach the final temperature of 48°C. Extra patties were treated in a similar manner as experimental controls. The temperature change of the pork was detected by a Thermistor digital temperature tester to ± 0.2% (Hantover, Kansas City, Mo) with a heavy duty meat probe. During heating, temperature of the thermal processing unit as well as temperature of the pork were recorded and the heating time was evaluated (Stumbo, 1965). Non-irradiated, heated samples were transferred to the cold storage as soon as the desired temperature was reached. Samples prepared for irradiation were placed in the insulated card-board boxes.

#### **Irradiation**

Ground pork was irradiated at the Linear Accelerator Facility of the Meat Laboratory, Iowa State University, at refrigeration temperature and at high

temperature in insulated styrofoam boxes. Temperature changes of ground pork before and after irradiation were recorded. During irradiation, the temperature of refrigerated samples was estimated to be between 4 and 5°C, and those of the heated samples were 45 to 46°C. A dose of 1 kGy was achieved in 4 min by adjusting the power level (1 or 3 kW) of the Linac and the conveyor speed (m/min). Absorbed doses were measured by placing 99% pure alanine pellets (Bruker Instruments, Inc., Billerica, MA) on the upper and the lower surfaces of the pre-packaged patties and the average dose used. An Electron Paramagnetic Resonance instrument (Bruker Analytische Messtechnik, Karlsruhe, Germany) was used to determine the free radicals formed and thus the absorbed doses. The instrument was calibrated with standard dosimeters traced to the National Standard by the National Institute of Standards and Technology (NIST).

### **Sensory Evaluation**

Pork samples were broiled on an electric stove until the internal temperature reached 68.3°C (155°F) as recommended by the American Meat Institute (Mermelstein, 1993). Meat temperature was detected by a Thermister digital temperature tester to  $\pm 0.2\%$  (Hantover, Kansas City, MO). Each patty was divided into 8 identical portions; and each portion was served for sensory evaluation at once. There were 8 samples representing 8 treatments for each panelist to evaluate. Panelists were untrained volunteer staffs and students of the Meat Science group. Their number ranged from 10 to 17 and they did not necessarily represent the same group of judges. A hedonic scale, 7 levels, were used to grade

aroma, flavor, tenderness, and juiciness. Score of 1 indicates dislike extremely, 7 indicates like extremely with 4 being neutral.

At the same panel session, panelists also scored the color and odor of the uncooked samples using similar scale of preference. No standard samples were used for comparison.

### **Physicochemical Measurements**

#### **Thiobarbituric Acid (TBA) and Hunter L, a, b values**

Lipid oxidation was determined in uncooked samples using the distillation 2-thiobarbituric acid (TBA) method described by Tarladgis et al. (1960). Color characteristics were measured as Hunter L, a, and b values (L=lightness, a=redness, b=yellowness) using a Hunter Labscan Spectrocolorimetry (Hunter Associates Laboratory, Inc., Reston, VA) as described by de Man (1990).

### **Statistical Analysis**

A factorial design of 2x2x2x5 was used. Main effects were packaging (air and vacuum), temperatures (2 and 48°C), irradiation (0 and 1 kGy), followed by 5 storage periods (0, 1, 2, 3, and 4 weeks). One sample was designated for each treatment and experiments were triplicates on different batches of pork.

Physicochemical analysis was performed on the same day of treatment applications. Sensory evaluation was carried out on the following day. Data were analyzed using the General Linear Model procedure (Statistical Analysis System, SAS, 1986).

## RESULTS AND DISCUSSION

### Sensory Evaluation of Raw Ground Pork

#### Odor

Irradiation resulted in significantly ( $p < 0.01$ ) lower odor scores, indicating less preference, in raw ground pork by panelists at week 0 (Figure 2). With extended storage to week 1, no significant ( $p > 0.05$ ) difference between irradiated and non-irradiated odor scores was found. At the end of week 2, however, mean odor scores of irradiated ground pork were significantly ( $p < 0.001$ ) higher than their non-irradiated counterparts. Generally, the odor scores of irradiated ground pork remained unchanged or slightly increased throughout the storage periods. In contrast, mean odor scores of non-irradiated samples progressively decreased, particularly after week 2, due to microbial spoilage (Navanugraha and Olson, 1996).

Zhao et al. (1995) reported a distinct irradiation odor initially developed on fresh pork chops treated at 1 kGy which disappeared after two weeks of storage. They also reported that continuously decreasing odor scores were found in non-irradiated pork after the first two weeks of storage at 2°C.

Lambert et al. (1992a) reported an irradiation-induced off-odor in 20% O<sub>2</sub> packed pork loins, 1 kGy irradiated at 0°C, which disappeared after 3-14 days of storage. They reasoned that the irradiation odor was dissipated upon storage at 5°C. They also reported that the microbial population was mainly lactic acid

bacteria (Lambert et al., 1992b) which produced less offensive odor as compared to the Gram-negative spoilage flora commonly found in non-irradiated fresh meat.

When protein-rich foodstuffs are irradiated, it is a common observation that off-odors and off-flavors are more pronounced immediately after irradiation than after a few days or weeks of storage (Diehl, 1983). This is not simply due to the evaporation of volatiles (Diehl, 1983) but rather the changes of radiolytic products upon prolonged storage.

Wick et al. (1967) applied gas chromatographic technique to study the radiolytic products on enzyme-inactivated irradiated beef (50 kGy) immediately after irradiation and after storage at ambient temperature for 6 months. They found that methional and n-alkanals, major volatile components of freshly irradiated beef, had almost disappeared in the stored samples which contained more n-alkanols than the fresh. Both freshly irradiated beef and the stored product contained n-alkanes and 1-alkenes, but these were present in much smaller quantity in the stored samples. This finding explains the possibility that irradiated components might have changed during storage.

Panelists preferred, in terms of odor, ground pork irradiated under vacuum than in air (Figure 2). Radiolytic products generated in the presence of oxygen were noted immediately after irradiation. Schweigert et al. (1954) suggested the exclusion of oxygen, vacuum packaging and sealing in the presence of "inert" gas, as a way to reduce the magnitude of the organoleptic changes induced by irradiation. The protective effect of vacuum packaging on the odor of ground pork

was present in our study but was not significant ( $p < 0.001$ ) until week 2 (Figure 2).

Panelists preferred the odor the ground pork treated at low temperature to high temperature at week 0 ( $p < 0.05$ ) and week 1 ( $p < 0.01$ ). Panelists might have related freshness to the acquainted odor of raw meat. Heating alters the fresh odor associated with raw meat. In the irradiated samples, irradiation at a higher temperature resulted in lower scores indicating a lower degree of liking. The less preferred odor scores were probably resulted from the off-odor developed by irradiation at a higher temperature which had tendency to cause an indirect effect of irradiation (Urbain, 1986).

#### **Thiobarbituric Acid (TBA) Measurement**

Irradiation caused a highly significant ( $p < 0.01$ ) increase in TBA values for all irradiated pork at week 0 except the heated/vacuum (HV/I) samples which remained unchanged (Figure 3). This trend of TBA values continued to week 1 ( $p = 0.05$ ). TBA values of ground pork, packaged in air and irradiated at 2°C (RA/I), progressively increased without interruption throughout the storage period while TBA values of irradiated, vacuum-packaged pork (HV/I, RV/I) remained unchanged. Vacuum packaging clearly protected ground pork from autoxidation during irradiation and prolonged storage at 2°C. Throughout the storage period, samples that were air-packaged and irradiated at 48°C (HA/I), remained lower in TBA values than their refrigeration treated (RA/I) counterparts. Irradiation at a higher temperature in air could have produced more free radicals that feed the autoxidation chain reaction than irradiation at a lower temperature. Combined effects of heating,

irradiation, and vacuum packaging appeared to work in concert to protect ground pork from oxidation. Panelists did not express a less preference odor scores to RA/I samples (Figure 2) even when the TBA values exceeded the threshold level of 1.0 mg/1000 g (Ehioba, 1987; Ehioba et al., 1987). Correlation analysis of odor and TBA values was not significant ( $p>0.05$ ) (Table 1).

### **Color**

Irradiation and packaging did not show an immediate effect on color in raw ground pork at week 0 (Figure 4). Heating resulted in significantly ( $p<0.05$ ) lower color scores on weeks 0, 1, and 2 and a highly significant ( $p<0.01$ ) increased L value at week 1 (Figure 5). Vacuum packaging significantly ( $p\leq 0.05$ ) decreased L values at week 1. Ground pork heated in oxygen-permeable packages, therefore, exhibited a paler color while ground pork heated in vacuum packages maintained the red color. Irradiation did not change the L, a, b values at any storage time (Figures 5, 6, and 7). Irradiation, however, caused a generally though not significantly ( $p>0.05$ ), lower color mean scores especially in air-packaged samples. Prolonged storage of vacuum packaged pork beyond week 1, followed by exposure to air (approximately 1 h) during the taste period, reversed the less preferred pork color induced by heating and irradiation (Figure 4). Panelists rated color of ground pork in vacuum packaging higher than in air at all combination of treatments. In general, the most preferred color was found in the HV/I treatment which was scored 5.8 at week 4. Color mean score in non-irradiated samples was  $5.3 \pm 1$  (mean  $\pm$  standard error of the means) on week 2. The samples, however, showed a shorter

storage life as evidenced by the psychrotrophic count of 6.8 log CFU/g (Navanugraha and Olson, 1996).

Zhao et al. (1995), after 1 kGy irradiation, found vacuum packaging to be superior to oxygen-permeable packaging in controlling the irradiation-induced undesirable color in pork chops. Their vacuum packaged samples resulted in irradiated pork color more similar to non-irradiated products and with slightly higher L and b values.

Lebepe (1989) and Lebepe et al. (1990) reported that pork cuts from vacuum-packed, irradiated (3kGy) loins, rewrapped with oxygen-permeable film had higher Hunter a values than chops from non-irradiated loins. Their Hunter L and b values, however, were inconsistent.

Correlation coefficients of color (panelists) with L and a values from Hunter Labscan were significantly correlated at  $p < 0.05$  and  $p < 0.001$  respectively (Table 2). This confirmed that panelists preferred a distinct fresh reddish color of pork to pale meat.

## **Sensory Evaluation of Cooked Pork**

### **Aroma and Flavor**

Aroma and flavor of cooked ground pork were not ( $p > 0.05$ ) affected by irradiation, temperature, and packaging methods (Figures 8 and 9). The less preferred odor associated with irradiated pork, found in uncooked products by panelists on week 0, was not continued after broiling. At weeks 0 and 1, irradiated pork scores were slightly lower in both aroma and flavor. By week 2, however, non-

irradiated samples were considered spoiled and received low scores while the non-irradiated samples were rated higher. Means of aroma and flavor scores for irradiated pork remained practically unchanged and in the preferable range up to weeks 3 and 4. At week 4, irradiated samples showed no signs of spoilage, by smell (off-odor) or touch (sliminess), except the refrigerated/air packaged samples. These samples exhibited a slight off-odor but no sliminess.

The correlations of odor/aroma and aroma/flavor are presented in Table 1. Aroma and flavor appeared to be positively correlated but not the odor and aroma. Cooking might have changed the nature of chemicals responsible for the "irradiated" odor found immediately after irradiation.

#### **Tenderness and Juiciness**

Irradiation at 1 kGy, as well as heating, did not ( $p>0.05$ ) have any impact on the texture and juiciness of ground pork at any storage time. Vacuum packaging, on the contrary, resulted in a more preferred pork in terms of tenderness and juiciness on weeks 1 ( $p<0.05$ ), 2 ( $p<0.05$ ), and 3 (Figures 10 and 11).

Muscle proteins are relatively resistant to irradiation. Most studies on meat texture have been performed in the radappertization dose range. There was no evidence that the radurization dose level as applied in this study (1 kGy), would alter the texture and water-holding-capacity in pork. When high dose irradiation was applied, Bailey and Rhodes (1964) observed increased solubility of collagen in irradiated ( $>10$  kGy) beef muscles resulting in tenderization of meat. However, Lawrie et al. (1961) reported the immediate effects of 50 kGy irradiation on beef and

pork *longissimus dorsi* muscles. They noted a decrease in water-holding-capacity and soluble protein, and increased resistance of the fibrils to homogenization suggesting cross-linking between protein molecules that toughens the meat.

Difference in the effect of radiation on tenderness of meat seems to lie in the difference in methods which incorporate heat treatments to inactivate enzymes in meat. In order to clarify this controversy, Whitehair et al. (1964) inactivated enzymes in pork using a high-temperature short-time (HTST) blanching with low-temperature long-time (LTLT) blanching prior to irradiation. They found meat to be tender in irradiated samples with HTST but no significant difference with LTLT treatment.

Heating in the thermal processing unit resembles the LTLT treatment. However, heating to a temperature of 48°C might have not completely destroyed the enzymes in meat. Neither heating nor irradiation, as employed in this study, was likely to sufficiently tenderize or increase the water-holding-capacity in meat when either treatment was applied alone.

Vacuum packaging has been known to increase purge loss in meat resulting in drier products (Urbain, 1983). However, sensory evaluation of tenderness and juiciness in this study indicated a preference for vacuum-packed pork over the air-packed (Figures 10 and 11). Panelists ranked the vacuum-packaged pork, irradiated at 45°C (HV/I) treatment, as the most preferred pork in terms of tenderness and juiciness.

## CONCLUSIONS

Irradiation at 1 kGy resulted in significantly ( $p < 0.01$ ) lower odor scores and increased thiobarbituric acid (TBA) values on week 0. Upon cooking and/or extended storage, no significant difference in the aroma ( $p > 0.05$ ) and the flavor ( $p > 0.05$ ) scores of ground pork was found at any storage times. TBA values were higher in irradiated pork, particularly in the air-packaged samples, irradiated at 2°C. At the end of the storage period, i.e. 4 weeks, TBA values of all irradiated samples were under the threshold level of 1.0 mg malonaldehyde/1000 g except samples from the RA/I (air-packaged, 1 kGy irradiation at 2°C) combination. TBA values of the RA/I treatment increased without interruption and exceeded the threshold level at week 1. TBA values from other treatments were below the threshold level. Radiation-induced oxidation at the refrigeration temperature was controlled by either vacuum packaging alone or heating alone or by the combination of the two.

Irradiation did not ( $p > 0.05$ ) cause a significant different effect on color, tenderness, and juiciness in ground pork at any storage time. Panelists scored vacuum packaged samples higher, in tenderness ( $p < 0.05$ ) and juiciness ( $p < 0.05$ ), after week 1 of storage. With extended storage time, vacuum packaging resulted in higher preference color scores, though they were not significantly different ( $p > 0.05$ ). The most preferred samples, for all attributes, resulted from vacuum packaging of pork followed by irradiation at 48°C.

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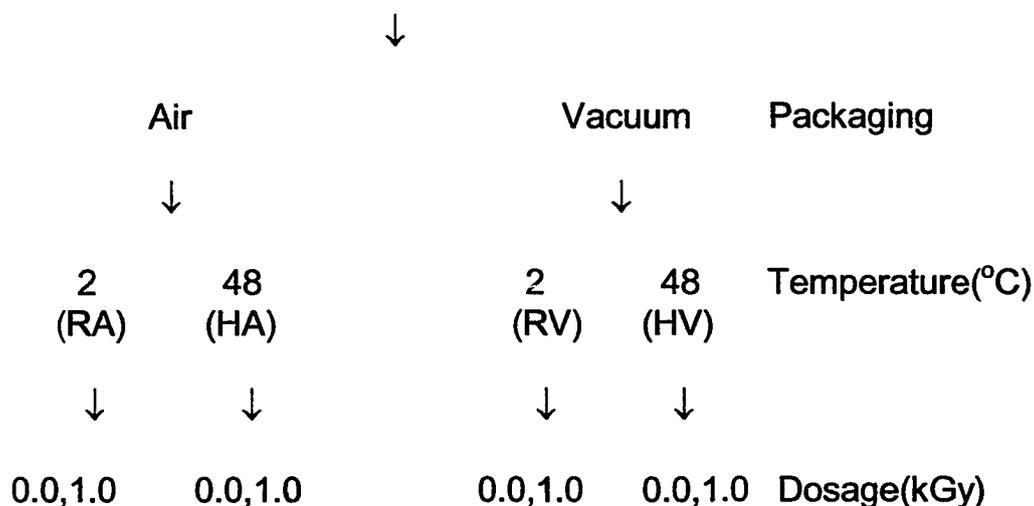
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Table 1. Correlation analysis of attributes in ground pork by panelists and by physicochemical methods

Correlated Attributes	Correlation Coefficient	P>F	N
aroma/odor	0.34	0.006	64
aroma/flavor	0.73	0.0001	64
odor/TBA	-0.17	0.1319	76
aroma/TBA	-0.61	0.0001	64
flavor/TBA	-0.51	0.0001	64
flavor/tender	0.43	0.0003	64
flavor/juice	0.37	0.0024	64
color/L	-0.31	0.0195	56
color/a	0.50	0.0001	56
color/b	-0.06	0.6819	56

## Ground Pork (~100g/each)



- Stored at 2°C for 0, 1, 2, 3, 4 weeks and analyzed weekly
- Physicochemical analysis:
  - Lipid oxidation---2-thiobarbituric acid (TBA)
  - Color---Hunter Labscan
- Sensory Evaluation (Taste Panelist):
  - Color, odor (Uncooked samples)
  - Flavor, aroma, tenderness, juiciness (Cooked samples)
- 7 point descriptive scale for sensory evaluation

RA = Refrigerated/Air      HA = Heated/Air  
 RV = Refrigerated/Vacuum      HV = Heated/Vacuum  
 N = Non-irradiated (0 kGy)      I = Irradiated (1 kGy)

Figure 1. Experimental design for physicochemical analysis and sensory characteristics of ground pork

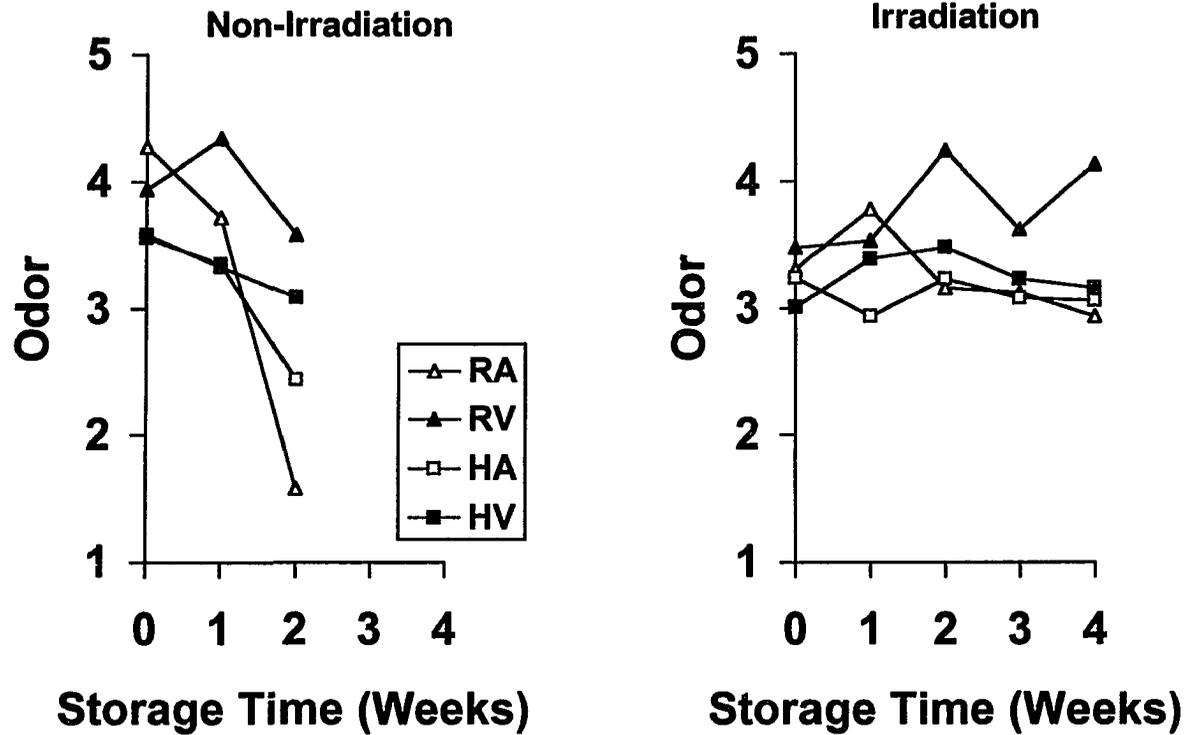


Figure 2. Effects of temperature of irradiation on odor of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.27, 0.26, 0.30, 0.16, 0.50 at week 0, 1, 2, 3, and 4 respectively)

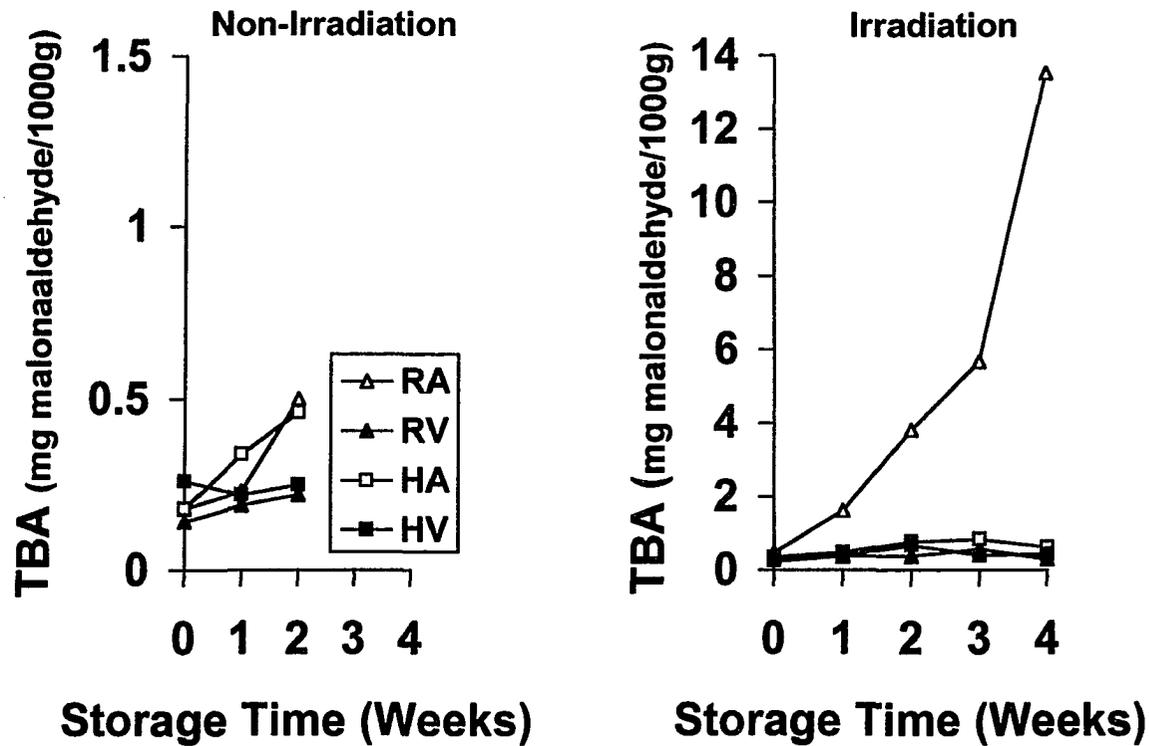


Figure 3. Effects of temperature of irradiation on TBA values of ground pork in vacuum and oxygen-permeable packaging stored at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.07, 0.30, 0.78 at week 0, 1 and 2 respectively; SEM was not available on weeks 3 and 4)

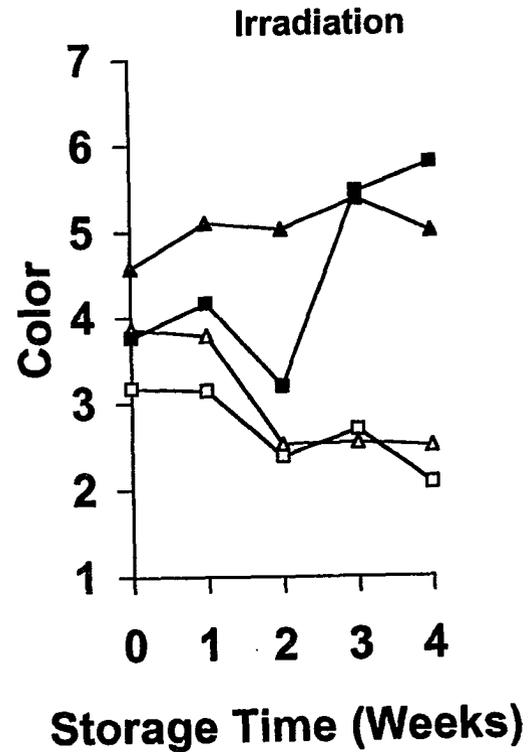
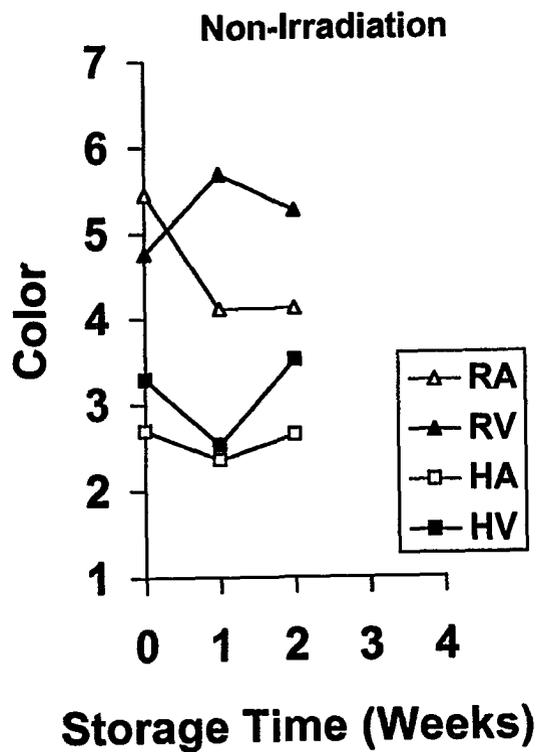


Figure 4. Effects of temperature of irradiation on color of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.65, 0.74, 0.85 at week 0, 1, and 2 respectively; SEM was not available on weeks 3 and 4)

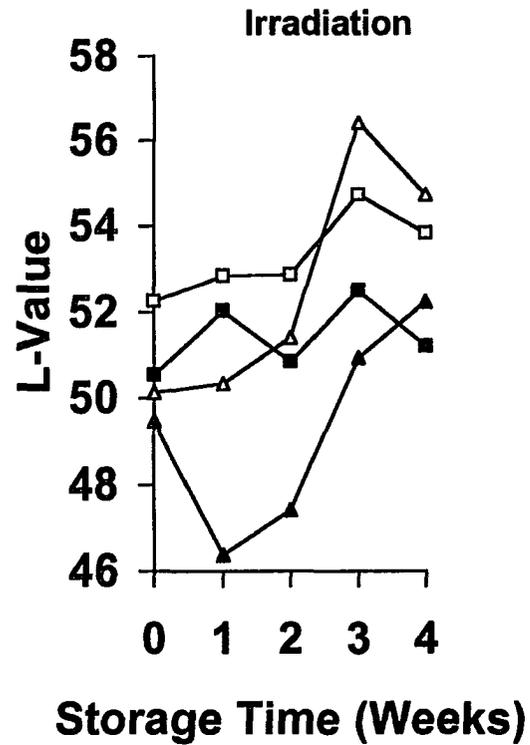
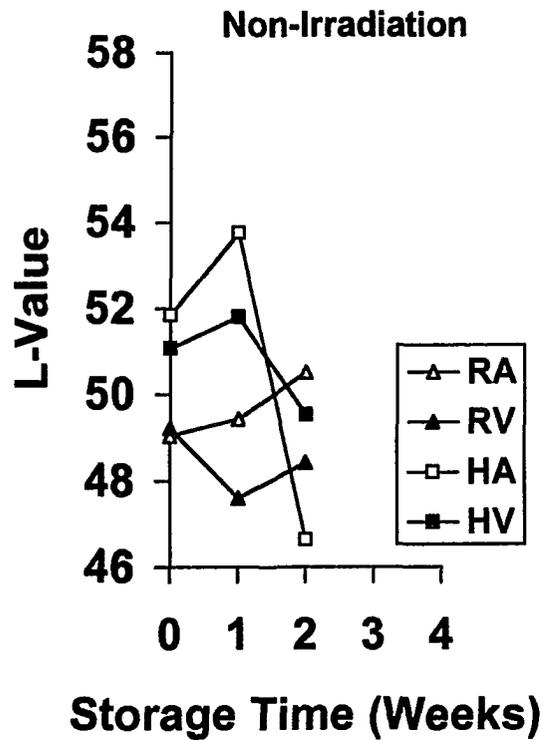


Figure 5. Effects of temperature of irradiation on L-values of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 1.60, 1.25, 1.78 at week 0, 1, and 2 respectively; SEM was not available on weeks 3 and 4)

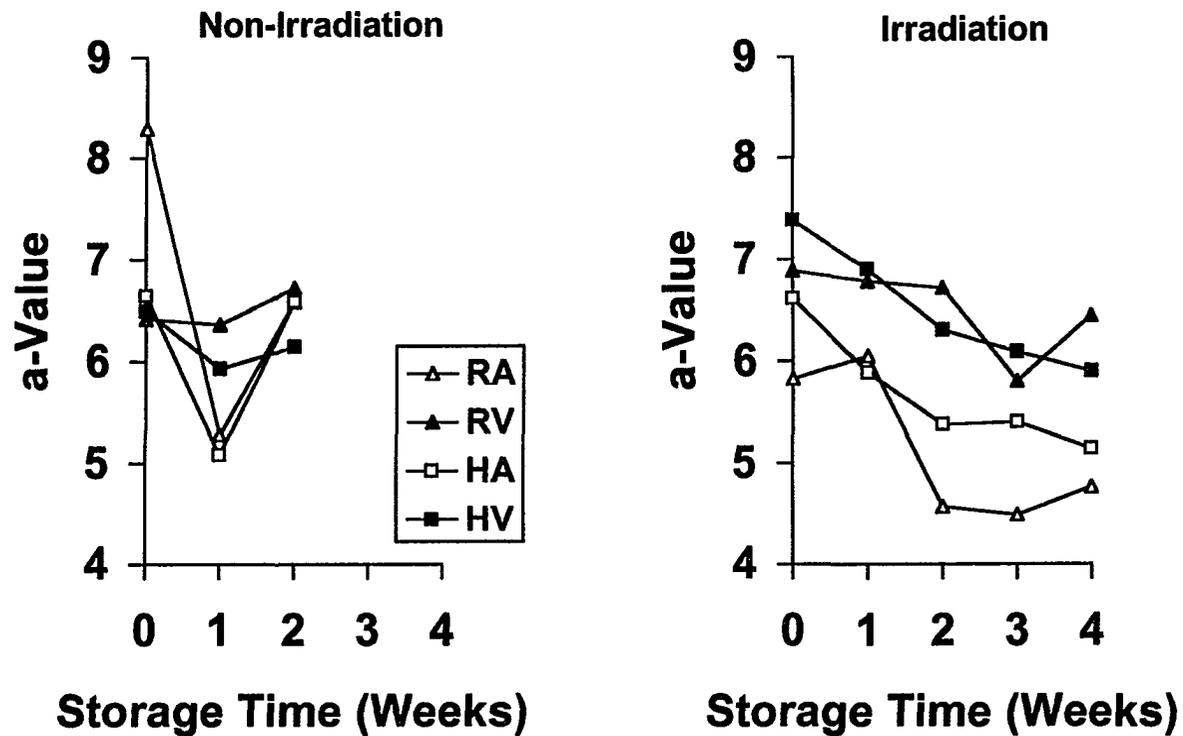


Figure 6. Effects of temperature of irradiation on a-values of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.63, 0.64, 0.65 at week 0, 1, and 2 respectively; SEM was not available on weeks 3 and 4)

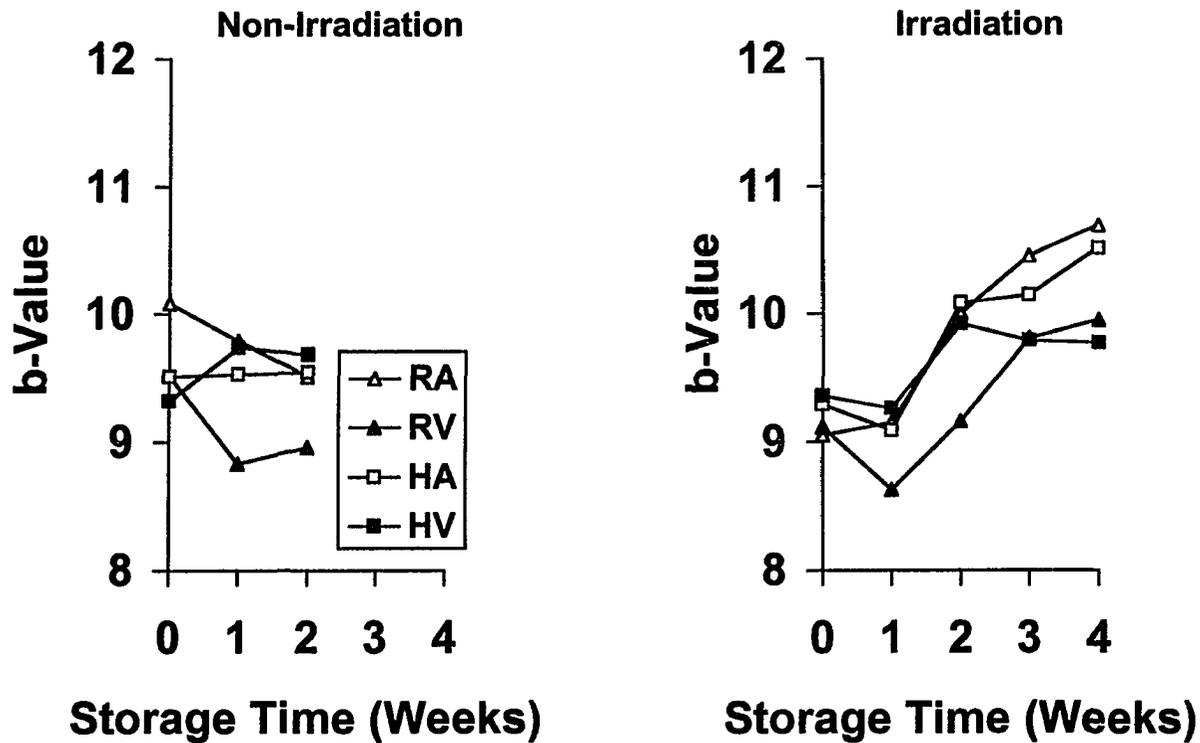


Figure 7. Effects of temperature of irradiation on b-values of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.35, 0.45, 0.64 at week 0, 1 and 2 respectively; SEM was not available on weeks 3 and 4)

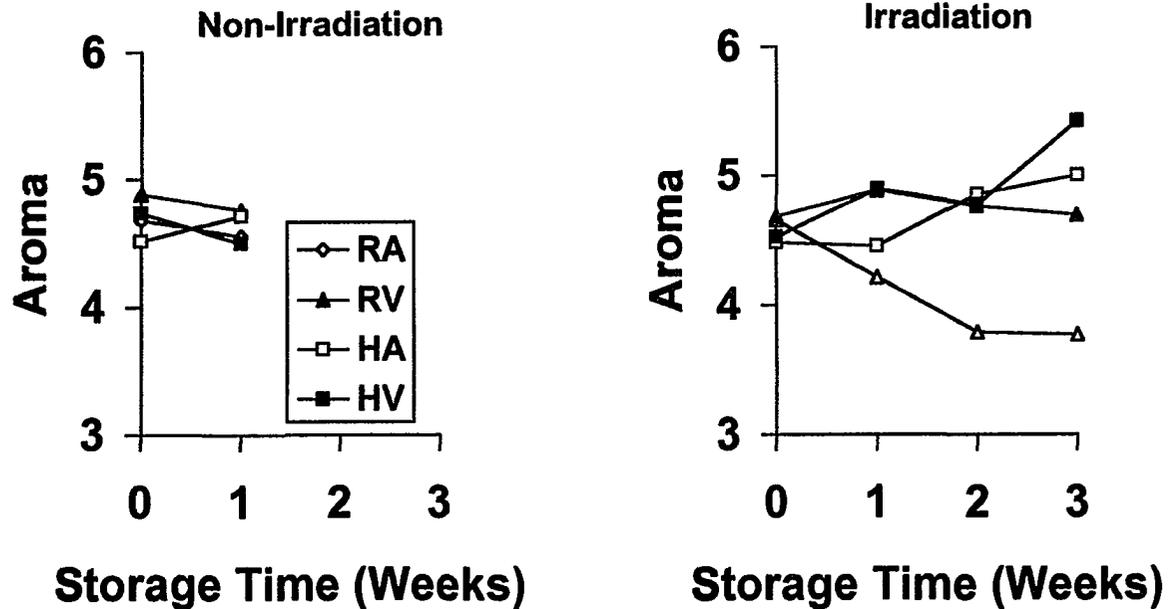


Figure 8. Effects of temperature of irradiation on the aroma of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.13, 0.26, 0.37 at week 0, 1 and 2 respectively; SEM was not available on weeks 3 and 4)

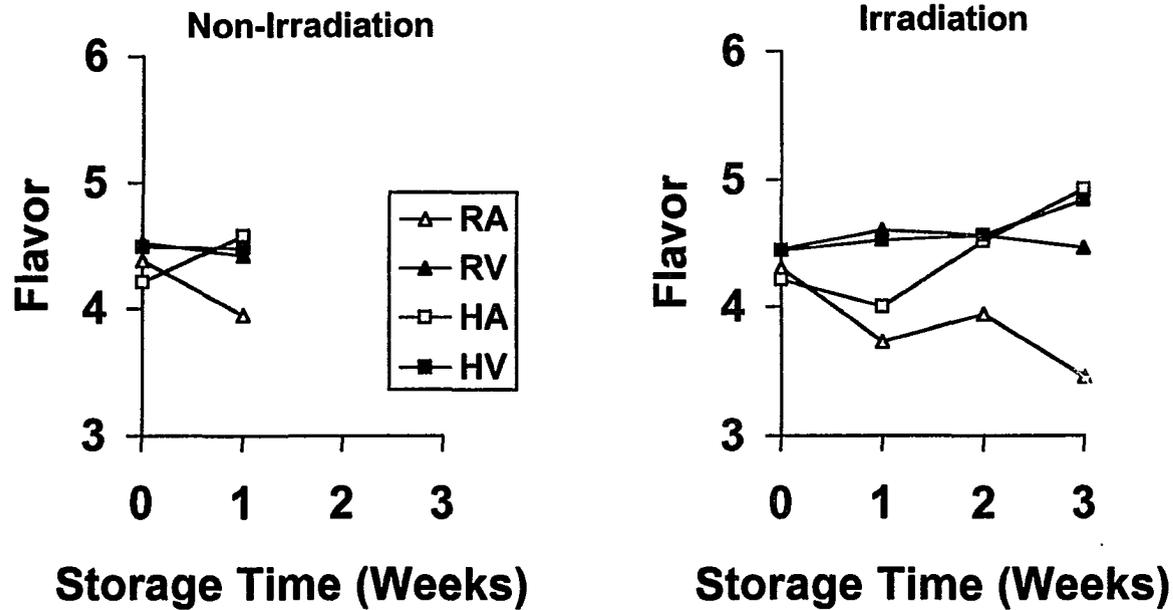


Figure 9. Effects of temperature of irradiation on the flavor of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.27, 0.31, 0.41 at week 0, 1 and 2 respectively; SEM was not available on weeks 3 and 4)

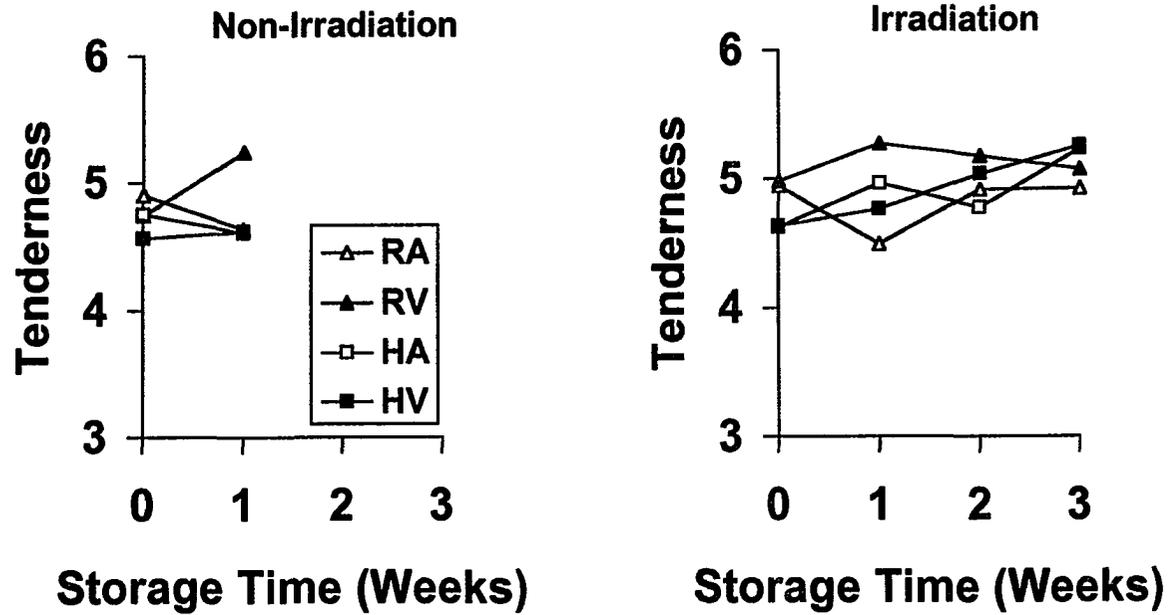


Figure 10 Effects of temperature of irradiation on the tenderness of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.18, 0.17, 0.15 at week 0, 1 and 2 respectively; SEM was not available on weeks 3 and 4)

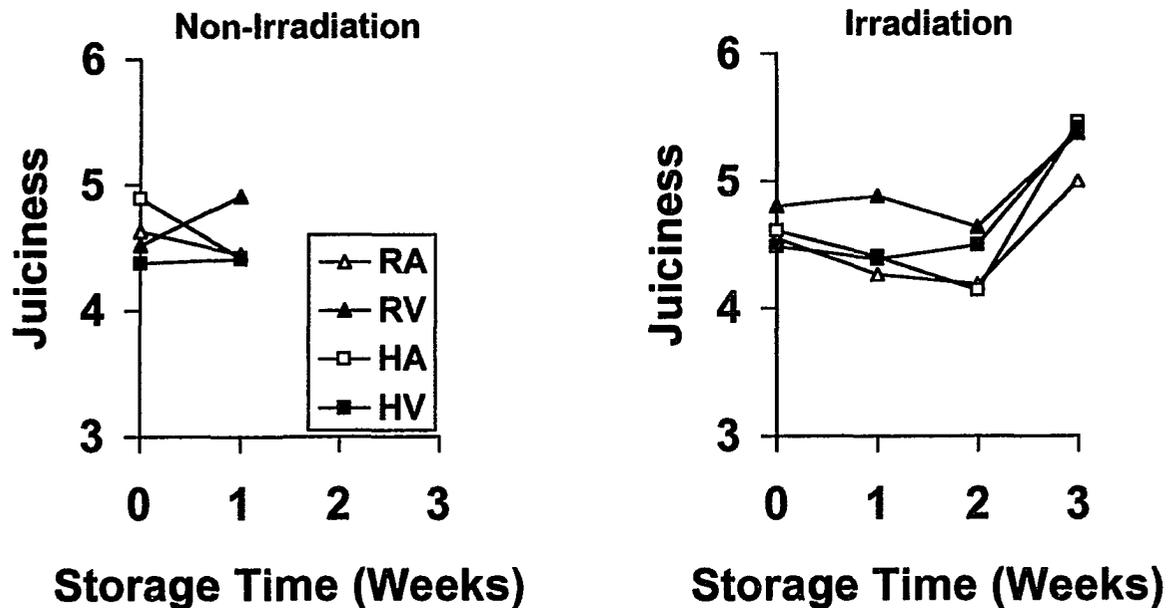


Figure 10 Effects of temperature of irradiation on the juiciness of ground pork in vacuum and oxygen-permeable packaging stored at at 2°C (RA=refrigeration, oxygen-permeable packaging; RV= refrigeration, vacuum packaging; HA=heat, oxygen-permeable packaging; HV=heat, vacuum packaging; SEM (Standard error of the means) = 0.25, 0.14, 0.15 at week 0, 1 and 2 respectively; SEM was not available on weeks 3 and 4)

### GENERAL SUMMARY

1. In the inoculated-pack study, *L. monocytogenes* Scott A was found in higher numbers in ground pork patties that were packaged under vacuum and recovered anaerobically than in air.
2. Within the dose range studied, irradiation at 48°C resulted in lower survivors than irradiation at 2°C. However, the rate of destruction at 2°C was higher than at 48°C. Consequently, irradiation D-values at 2°C were lower than at 48°C.
3. Pork patties that were vacuum packaged and irradiated at 48°C appeared to show the longest storage-life (~35 days).
4. Panelists scored irradiated samples lower than non-irradiated samples, in terms of odor, when the taste panel was performed within 24 h after irradiation. This low score was discontinued in broiled pork, or in pork stored for one week.
5. Vacuum packaging resulted in higher scores for tenderness and juiciness from after prolonged storage, i.e. after week one.
6. Oxidative rancidity, as expressed by 2-thiobarbituric acid, was highest in air packaged pork, irradiated at 2°C. This, however, did not result in lower sensory scores of these samples in either uncooked or broiled state.
7. Sensory scores of irradiated, cooked, ground pork were not significantly different ( $p>0.05$ ) from those of non-irradiated pork in terms of color, aroma, flavor, tenderness, and juiciness.

**Recommendation for Future Work**

Irradiation-induced and oxidation-induced off flavor/odor should be evaluated at various doses of irradiation in parallel to the response from taste panelists. At present, the "irradiated" flavor, particularly at radurization doses, has not been extensively investigated. There is a controversy about the nature of irradiated flavor and oxidative flavor which appears to come from different components of foods. Some researchers believe that oxygen content during irradiation affect the flavor of irradiated foods while others believe otherwise. There appears to be two processes involved in the irradiation of foods, namely, physical processes which occur instantaneously (approximately within  $10^{-11}$  sec) and the chemical processes that follow and remain in effect throughout the storage time (an after-effect). It is the chemical reactions that result in many undesirable changes in irradiated foods. Research in this field may explain the nature of irradiated flavor in foods that will help us find the best method to extend the storage-life of food while maintaining its quality.