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Effect of irradiation and antimicrobials on the growth and survival of Listeria monocytogenes and quality of ready-to-eat turkey meat products

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Effect of irradiation and antimicrobials on the growth and survival of *Listeria monocytogenes* and quality of ready-to-eat turkey meat products

by

Meijun Zhu

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For the Major Program
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This dissertation investigated the effect of irradiation and antimicrobials on the quality and survival of L. monocytogenes, a significant safety concern, in RTE turkey meat products. Results showed that dietary vitamin E was effective in reducing the number of L. monocytogenes carried by live turkeys, a source of L. monocytogenes contamination in RTE products. Irradiation was very effective in eliminating L. monocytogenes in both turkey breast rolls and hams. However, irradiation also caused quality changes. A dose of 2.0 kGy irradiation produced significant off-odor, mainly sulfur-related odor/flavor, in vacuum-packaged RTE turkey ham and rolls. Thus, only low dose irradiation is recommended. However, low-dose irradiation allowed some L. monocytogenes to survive during irradiation. Antimicrobial agents were formulated into RTE turkey rolls and hams as an additional hurdle to suppress the growth of L. monocytogenes that survived low-dose irradiation. Antimicrobials, including 0.1% potassium benzoate (PB), 2% sodium lactate (SL), and 0.1% sodium diacetate (SDA) were used singly or in combinations to assess their effectiveness in inhibiting the growth of L. monocytogenes in RTE meat products following low-dose irradiation. Results showed that for RTE turkey meats formulated with 0.1% PB+2% SL or 2% SL+0.1% SDA, 1.0 kGy irradiation was effective in suppressing the growth of L. monocytogenes for about six weeks at 4 °C, and 2.0 kGy irradiation was listeriostatic during a 42-day refrigerated storage period. For RTE turkey meat formulated with three antimicrobials in combination, 0.1% PB+2% SL+ 0.1% SDA, 1.0 kGy irradiation was listeriostatic. Including 2% SL in formulation, however, slightly affected the texture and color values of turkey breast rolls, and the addition of PB in RTE turkey meats produced a high amount of benzene after irradiation. Considering their antilisterial activity and their effects on quality, including 2% SL plus 0.1% SDA in formulation in combination with 1.0- or 2.0 kGy-irradiation was a promising technology to ensure the safety of RTE turkey ham and breast rolls from L. monocytogenes.

Keywords: Listeria monocytogenes; e-beam irradiation; antimicrobial; quality; ready-to-eat; turkey breast roll; turkey ham
CHAPTER 1. GENERAL INTRODUCTION

Listeria monocytogenes is a major human bacterial food-borne pathogen that annually accounts for ~2,500 cases (meningitis, encephalitis, sepsis, fetal death, premature birth) and 504 deaths (Mead et al., 1999). Sporadic human cases of listeriosis have been epidemiologically linked to the consumption of undercooked poultry products (Schwartz et al., 1988; Schuchat et al., 1992). A 1998 multistate outbreak of human listeriosis, ascribed to serotype 4 (101 cases, resulting in 22 deaths), was linked to delicatessen meats, including turkey (MMWR, 1998). A 2002 outbreak involving 46 cases, seven deaths, and three stillbirths was linked to contaminated delicatessen turkey meats (MMWR, 2002). The recall of 26 million pounds of turkey meat in 2002 indicates the economic consequences of ready-to-eat meats contaminated with L. monocytogenes (U.S. Department of Health and Human Services, 2002). Therefore, a successful strategy is urgently needed to prevent the contamination and subsequent proliferation of L. monocytogenes in RTE turkey meat products.

An important source of L. monocytogenes contamination in turkey meat products is the live turkey. L. monocytogenes may enter the packing plant at low levels in the intestine of recently infected birds, survive in bio-films, and ultimately contribute to both environmental and ready-to-eat product contamination (Genigeorgis et al., 1990; Ojeniyi et al., 1996). Since Vitamin E supplement enhances the immune response in chicken, it may be effective in reducing L. monocytogenes carried by live birds. Dietary vitamin E supplementation was tested for its effectiveness in accelerating the gut clearance of L. monocytogenes in experimentally infected adult turkeys. Results showed that vitamin E supplementation was effective in boosting immune response and accelerating the elimination of inoculated L. monocytogenes in live turkeys (Zhu et al., 2003a).

Although L. monocytogenes can be killed during the thermal processing of RTE meats
(Lemaire et al., 1989; Zaika et al., 1990), post processing contamination of RTE meat with *L. monocytogenes* during slicing and packaging is difficult to avoid, due to its ubiquitous nature. Thus, it is necessary to have strategies to control or eliminate those products contaminated with *L. monocytogenes* in RTE meat. Irradiation and antimicrobials are best choices for this purpose, but their effects on quality and survival of *L. monocytogenes* in RTE turkey products have not been studied systemically. Hence, the effect of irradiation and antimicrobials on the quality and survival of *L. monocytogenes* in RTE turkey products are studied in this research.

Irradiation can induce meat quality changes such as off-odor and color change, and consumer responses to these quality changes are quite negative (Patterson & Stevenson, 1995; Ahn et al., 2000; Jo & Ahn, 2000; Du et al., 2002). Most of the irradiation studies so far, however, are mainly focused on raw meat and cooked meat without additives, and little information on the quality changes of RTE meat products by irradiation are available. Therefore, the effect of irradiation on the volatiles, sensory characteristics, color, and lipid oxidation of RTE turkey ham and breast rolls were evaluated. Results showed that irradiation caused off-odor/flavor in RTE turkey products. To prevent the quality change, a low-dose of irradiation is preferred. Thus, the effect of low dose irradiation on the survival of *L. monocytogenes* was further examined. Results showed that e-beam irradiation was effective in eliminating artificially contaminated *L. monocytogenes* in RTE turkey meats. However, low-dose irradiation, which is necessary to prevent quality change, was not sufficient to eliminate contaminated *L. monocytogenes*. Some *L. monocytogenes* that survived can repair themselves and can cause a health hazard during refrigerated storage (Gursel et al., 1997; Foong et al., 2004), indicating that additional hurdles are needed to prevent the survived *L. monocytogenes* from growth in RTE meat products.

Antimicrobials are good choices for this purpose. Sodium lactate, sodium diacetate, and potassium benzoate are extensively used to extend the shelf-life and ensure the safety of food products (Samelis et al., 2001; Meyer et al., 2003; Choi and Chin, 2003). However, little
is known about their effects in combination with irradiation on the quality and survival of *L. monocytogenes* in RTE turkey meat products. Thus, 0.1% potassium benzoate (PB), 2% sodium lactate (SL) and 0.1% sodium diacetate (SDA) were formulated singly or in combinations into RTE turkey meats, and their effectiveness in suppressing the growth of *L. monocytogenes* in RTE turkey breast rolls and hams after irradiation were evaluated. Results showed that low-dose irradiation at 1.0 or 2.0 kGy in combination with antimicrobials were very effective in controlling the growth of *L. monocytogenes* in RTE meat products. To successfully apply antimicrobials and irradiation to turkey RTE meats, it is necessary that they do not have significant negative effects on meat quality. Thus their effects on the quality of turkey breast rolls and hams were further evaluated. We found that antimicrobials had no major negative effects on meat quality. Combining low concentration of antimicrobials with low-dose irradiation was a potential strategy to ensure the safety of RTE meat products from *L. monocytogenes*. 
Dissertation organization

This dissertation is composed of a general introduction (Chapter 1), literature review (Chapter 2), 7 individual papers (Chapter 3-9) and a general conclusion (Chapter 10). The literature review summarizes the recent progress in the research about *Listeria monocytogenes* contamination associated with RTE meat products and possible intervention technologies, and the effect of irradiation and antimicrobials on the quality of meat product. Chapter 3 is about the effect of dietary vitamin E on the *L. monocytogenes* infection in turkeys. Chapter 4 and 5 show the effect of irradiation on the quality of RTE turkey ham and breast rolls. Chapter 6 is about the effect of irradiation on the survival of *L. monocytogenes* in vacuum packaged turkey ham and breast rolls without antimicrobial additives. Chapter 7 reflects the quality changes in turkey breast rolls induced by irradiation and antimicrobials, and Chapter 8 indicates the survival of *L. monocytogenes* after irradiation in turkey breast rolls formulated with antimicrobials. Chapter 9 reports on the quality change and the survival of *L. monocytogenes* in irradiated turkey hams formulated with antimicrobials. Chapter 10 is the general conclusion of this dissertation.
CHAPTER 2. LITERATURE REVIEW

Part I. Listeria Monocytogenes and Food Safety

1. Listeriosis, a major food-borne disease associated with ready-to-eat (RTE) meat

1.1 Listeria monocytogenes and Listeriosis

Food borne diseases caused by contaminated foods are one of the most widespread problems (Schlech, 1991; Notermans and Hoogenboom-Verdegaal, 1992). Listeria monocytogenes is one of the most frequent pathogens causing food borne diseases; Listeriosis annually accounts for about 2,500 cases and around $200 million monetary loss. Because of its high mortality rate (~ 25%) (Mead et al., 1999) and economic impact due to products recall (FSIS, Oct. 17, 2003), L. monocytogenes is still a major food safety issue for the processed meat industry.

L. monocytogenes is a gram-positive, non-sporeforming, highly mobile, rod-type, facultative anaerobic bacterium (Farber and Peterkin, 1991). It can grow from 0 to 42 °C (Ralovich, 1992) and the pH range for growth is between 4.5 and 9.6 (Seelinger and Jones, 1986). The organism generally grows well in meats near or above pH 6 and poorly or not at all on meat products near or below pH 5 (Glass and Doyle, 1989). It tolerates salt and nitrite (McClure et al., 1997). It is widely present in plant, soil, silage, sewage, slaughterhouse waste, milk, human and animal feces, and processing environments (Farber and Peterkin, 1991). They may form biofilm on different surfaces. Their growth kinetics, persistence and heat resistance can be influenced by many factors including the type of growth environment, pH, acidulants, salts, chemicals, antibiotics, plant substances, enzymes, humidity, atmosphere, temperature, prior temperature-effects, and microbial competition (Ralovich, 1992).

Due to its ubiquity in the environment, it is a challenge to prevent the transmission of L. monocytogenes from raw animal products to meat processing environment and to RTE meats (Beresford et al., 2001). Further, L. monocytogenes that contaminates cured and non-cured
RTE meat can proliferate in these products during refrigerated storage due to its ability to grow in the presence of curing salt at refrigerated temperature (Lou and Yousef, 1999). The emergence of multiple resistance in Listeria spp., due to acquisition of a replicon originated from staphylococci, further heightens the concern (Lemaitre et al., 1998). Thus, L. monocytogenes is one of the major food safety problems, especially for RTE meats, which usually have long shelf-life and consumed without further heating.

There have been three well-publicized outbreaks of listeriosis involving RTE meat products. A multistate outbreak in 1998 to 1999 was linked to frankfurters and deli meats and caused 101 cases and 21 deaths [Centers for Disease Control (CDC), 1999]. In 2000, a multistate outbreak involving deli turkey meat resulted in 29 cases, 4 death and 3 miscarriages or still birth (CDC, 2000). More recently, a multistate outbreak of L. monocytogenes infections in the Northeast United States was attributed to the consumption of sliceable turkey deli meat. There were 46 confirmed cases, 7 deaths and 3 still births or miscarriages associated with this outbreak (CDC, 2002). These reports clearly implicate that RTE meat products, especially poultry products, are important sources of listeriosis. The recall of 26 million pounds of turkey meat in 2002 indicates the economic consequences of RTE meats contaminated with L. monocytogenes (U.S. Department of Health and Human Services, 2002).

1.2. Incidences of Listeriosis and mechanisms of pathogenesis

Listeriosis is found mainly among pregnant women and their fetuses, immunocompromised individuals (AIDS, cancer), and elderly (over 60 years old), with symptoms of abortion, septic premature birth, neonatal death, septicemia, and meningitis (Farber and Peterkin, 1991; Schwarzkopf, 1996; Vazquez-Boland et al., 2001). In 1986-87, CDC conducted an active population-based survey of L. monocytogenes infections in six regions of the United States. Listeriosis was reported in 154 patients: one-third of the cases were perinatal and the remaining two-thirds occurred in the elderly and immunosuppressed.
Twenty eight percent of the cases were fatal (Schwartz et al., 1988). In addition to humans, *L. monocytogenes* affects many vertebrate species, including birds. *Listeria ivanovii*, a second pathogenic species of the genus, is specific for ruminants (Vazquez-Boland et al., 2001).

*L. monocytogenes* is an invasive bacterial pathogen capable of multiplying inside many host cells, including macrophages, enterocytes and hepatocytes (Berche et al., 1988). By invading and locating itself intracellularly in a site where host defense mechanisms are minimal, it escapes phagosome and enters the cytoplasm, from which it travels to the next cell without exposing itself to extracellular environments (Campbell, 1994). Pathogenic listeriae enter the host primarily through the intestine. Liver is thought to be the first target organ after intestinal translocation. In liver, *listeriae* actively multiply until the infection is controlled by a cell-mediated immune response (Vazquez-Boland et al., 2001). *Listeriae*, using an actin-based motility process to enter cells, escape from the phagocytic vacuole and spread from one cell to another (Cossart and Lecuit, 1998). Numerous extracellular and cell-associated proteins are involved in *L. monocytogenes* entry into mammalian cells, survival inside the phagosome, escape into cytoplasm, and cell-to-cell spread (Bhunia, 1997). Among them, Listeriolysin O and Act A are critical for the infection process (Cossart and Lecuit, 1998). Act A induces actin polymerisation at one pole of the bacterium and promotes intracellular and intercellular motility (Skoble et al., 2000; Cossart and Bierne, 2001; Portnoy et al., 2002). The secreted pore-forming toxin listeriolysin O, an SH-activated exotoxin, promotes the escape of *L. monocytogenes* from the phagocytic vacuole (Berche et al., 1988; Cossart and Lecuit, 1998; Portnoy et al., 2002), which is a major factor promoting intracellular growth of *L. monocytogenes* (Berche et al., 1988). The intracellular growth of virulent bacteria is initiated after escaping from the phagosomal compartment (Berche et al., 1988).

### 1.3. Immune responses to Listeriosis

The *Listeria monocytogenes*-carrying rates were 100% for listeriosis patients and 1.3%
for healthy humans (Iida et al., 1998). The risk of a healthy individual developing listeriosis after consumption of a contaminated product is very low, which supports the idea of indirect vaccination with less virulent strains (Schwarzkopf, 1996). However, the risk group can probably get listeriosis after eating food contaminated with even a few bacteria. T-cells are important in host response to *listeriae* infection (Roark et al., 1996; Wing and Gregory, 2000). Memory CD8⁺ T-cells is crucial in early protection and promotion of Th1 response against a primary infection by *L. monocytogenes* (Yajima et al., 2001). As a part of the immune response during acute listeriosis, the host marshalls neutrophils, macrophages, natural killer cells, and T-lymphocytes, especially CD4⁺ and CD8⁺ (Unanue, 2002).

However, the mechanisms by which CD8⁺ T-cell-mediated immunity against *listeria* remain largely unknown. Perforin-dependent cytolysis plays a role, but is not required for CD8⁺ T-cell-mediated immunity against *L. monocytogenes*. Tumor necrosis factor (TNF) is essential for CD8⁺ T-cell immunity to *L. monocytogenes* (White et al., 2000). Shortly after *Listeriae* infection, increase in the amounts of mRNA for TNF-alpha, interleukin-1 alpha (IL-1 alpha), IL-1 beta, and IL-6 were detected in P388D1 mouse macrophages. The induction of these cytokine mRNAs requires entry of *listeriae* into host cell cytoplasm (Kuhn and Goebel, 1994). IL-8 plays a role in neutrophil-mediated host defense during the acute phase of *L. monocytogenes* (Arnold and Konig, 1998).

2. **Source of *L. monocytogenes* contamination**

*L. monocytogenes* is commonly found in natural environments, the intestinal tract of infected animals, food processing environments and catering facilities (Autio et al., 2000; Gillespie et al., 2000; Beresford et al., 2001). An important source of contamination is through faeces that may come from animal feed. Adult animals may be transiently colonized by consuming contaminated feed or water (Husu et al., 1990). Thus, *L. monocytogenes* may enter packing plants at low levels in the intestine of recently infected animals. Some *Listeria*
monocytogenes strains may survive in bio-films, persist in processing environments, and ultimately contribute to both environmental and RTE product contamination (Giovannacci et al., 1999; Berrang et al., 2002). Fenlon et al. (1996) reported that the excretion of L. monocytogenes by farm animals was linked to their diet. Animals fed entirely on hay or manufactured diets did not excrete detectable levels of Listeria. However, animals fed on silage, which is frequently contaminated with L. monocytogenes, commonly excreted the organism. Transport of live animals over long distances (> 100 km) significantly increased the excretion of Listeria, and processing significantly increased the level of contamination (Fenlon et al., 1996).

2.1. L. monocytogenes in live poultry and poultry products

Poultry products are susceptible to Listeria contamination (Rorvik et al., 2003). Sporadic human cases of listeriosis have been epidemiologically linked to the consumption of undercooked poultry products (Schwartz et al., 1988). In the United States, L. monocytogenes was found in 5.9% of turkey carcass rinses and in 31% of ground turkey meat examined in the Nationwide Young Turkey Microbiological Baseline Data Collection Program (USDA-FSIS, 1998). In Berlin, out of 100 chicken (95 broilers, 5 layers), L. monocytogenes was isolated from 85 carcasses (Schonberg et al., 1989). In Northern Ireland, Listeria spp. were present in 38 of 80 retail packs of raw chicken chosen from supermarkets (48%) and 14 (18%) of them yielded L. monocytogenes (Soultos et al., 2003). In Finland, a study showed that 62% (38 of 61) of raw broiler pieces bought from retail stores were positive for L. monocytogenes (Miettinen et al., 2001).

2.2. L. monocytogenes in red meats

A report in Japan showed that the contamination rates of L. monocytogenes for retail sliced beef (34.2%) and pork (36.4%) were significantly higher (p < 0.05) than those for cattle (2.0%) and pigs (0.8%), and for cattle (4.9%) and swine (7.4%) carcasses (Iida et al.,
1998). To determine the extent of microbiological contamination in pork in the U.S., 384 samples of retail pork were collected from 24 stores in six cities, and additional samples (n = 120) of freshly ground pork and/or pork sausage were collected from two hot-boning sow/boar sausage plants, two slaughter and fabrication plants, and two further-processing plants. *L. monocytogenes* was detected in 26.7% of plant samples and 19.8% of retail samples, and was present more frequently in ground products (Duffy et al., 2001). In Finland, ten low-capacity slaughterhouses were examined for *Listeria* by collecting a total of 373 samples, of which 50, 250, and 73 were taken from carcasses, pluck sets, and the slaughterhouse environment, respectively. Six of 50 (12%) carcasses were contaminated with *L. monocytogenes*. Of the samples taken from pluck sets, 9% were positive for *L. monocytogenes*, the highest prevalence occurring in tongue and tonsil samples, at 14% and 12%, respectively (Autio et al., 2000). *L. monocytogenes* of tongue and tonsil origin may contaminate the slaughtering equipment that may in turn spread the pathogen to carcasses (Autio et al., 2000).

### 2.3. *L. monocytogenes* in RTE meats

Ready-to-eat meat products are the most popular meat products in the U.S. However, RTE meat products are frequently contaminated with *L. monocytogenes* mostly due to post-processing contamination. Higher incidence rates were obtained for whole cooked meat products (e.g. cooked ham, bacon) after slicing than before slicing, indicating cross-contamination. Due to multiple handling and processing steps, the incidence rate of pathogen contamination was higher for cooked minced meat products than for whole cooked meat products (Uyttendaele et al., 1999).

*L. monocytogenes* were isolated from 10 out of 68 "oven-ready" poultry purchased at 26 different shops and supermarkets (Gitter, 1976). A report about *L. monocytogenes* in nine different categories of RTE meat and poultry products for the years 1990 through 1999 showed that the cumulative 10-year *L. monocytogenes* prevalences were as follow: jerky,
0.52%; cooked, uncured poultry products, 2.12%; large-diameter cooked sausages, 1.31%; small-diameter cooked sausages, 3.56%; cooked beef, roast beef, and cooked corned beef, 3.09%; salads, spreads, and pates, 3.03%; and sliced ham and luncheon meat, 5.16%. The cumulative 3-year *L. monocytogenes* prevalence for dry and semidry fermented sausages was 3.25% (Levine et al., 2001). Of the random FSIS samples collected and analyzed between Jan. 1 and Sept. 30, 2003, 0.75 percent of RTE meats tested were positive for *L. monocytogenes* (Food Safety and Inspection Service, 2003).

### 2.4. *L. monocytogenes* in other foods

*L. monocytogenes* has been found in a wide range of dairy products such as raw milk and varieties of cheeses (Farber and Peterkin, 1991; Mickova, 1992; Cordano and Rocourt, 2001). The contamination of *L. monocytogenes* in raw cured meat products was significantly higher than that of cooked meat products (Uyttendaele et al., 1999). A high incidence rate of *L. monocytogenes* was noted for mayonnaise based salads as well as for prepared meals (Uyttendaele et al., 1999). At Maryland and northern California FoodNet sites, eight categories of RTE foods (luncheon meats, deli salads, fresh soft "Hispanic-style" cheeses, bagged salads, blue-veined and soft mold-ripened cheeses, smoked seafood, and seafood salads) were collected over 14 to 23 months from retail markets. Of 31,705 RTE food samples tested, 577 were positive. The overall prevalence was 1.82% (Gombas et al., 2003).

### 2.5. *L. monocytogenes* contamination in processing plants

Processing facilities are important sources of *L. monocytogenes* contamination. *L. monocytogenes* were tested for their ability to adhere to stainless steel. Significant differences exist in the ability of various *L. monocytogenes* strains to attach to a test surface. In monoculture, the majority of strains did not form biofilms (Kalmokoff et al., 2001). Preculturing *L. monocytogenes* in a lactic acid-supplemented medium increased the affinity of microbial cells to solvents and the bacterial attachment to stainless steel (P < 0.05)
(Briandet et al., 1999). The *L. monocytogenes* biofilms attached more strongly to polymers than other pathogens such as *Staphylococcus sciuri*, *Pseudomonas putida*, or *Comamonas sp.*, and the attachment strength was weaker on stainless steel than on polymers (Midelet and Carpentier, 2002). Dust contaminated with *L. monocytogenes* was another source of contamination (De Roin et al., 2003).

Contamination analysis of persistent and nonpersistent *L. monocytogenes* strains in three meat processing plants and one poultry processing plant were performed by Finland researchers. Persistent and nonpersistent strains were found in all plants. Nonpersistent strains were found mostly at one sampling site, with the processing environment being the most common location, whereas the persistent strains were found at several sampling sites in most cases. The processing machines were frequently contaminated with persistent *L. monocytogenes*, which play an important role in contaminating products (Lunden et al., 2003). The contamination status of processing lines and machines appeared to be influenced by the compartmentalization of the processing line, with poor compartmentalization increasing *L. monocytogenes* contamination (Lunden et al., 2003).

In Northern Ireland, the incidence of *Listeria spp.* and *L. monocytogenes* in a poultry processing plant was determined over a 6-month period. Within raw and cooked poultry processing environments, 46% (36 of 79) and 29% (51 of 173) of the samples contained *Listeria spp.* while 26% (21 of 79) and 15% (27 of 173) contained *L. monocytogenes*, respectively. Various sites within the processing environment were found to be consistently positive for *L. monocytogenes* throughout the sampling period (Lawrence and Gilmour, 1994). In Finland, the environment and products from two broiler abattoirs and processing plants and raw broiler pieces at retail level were sampled for *L. monocytogenes*. At the broiler abattoir, air chiller, skin-removing machine, and conveyor belt leading to packaging area were positive for *L. monocytogenes*. The *L. monocytogenes* contamination rates varied from 1 to 19% between the two plants studied. The same PFGE types found in raw broiler pieces at retail level were also found in broiler abattoirs where the broilers had been slaughtered.
3. **Intervention of L. monocytogenes contamination**

Heating is effective in destroying *L. monocytogenes*, thus *L. monocytogenes* contaminated meat could be virtually eliminated during the cooking step of RTE meats processing. Therefore, *L. monocytogenes* contamination in RTE meats is mostly due to post-heating contamination. The post-package decontamination, such as in-package thermal pasteurization and irradiation, and formulating meat products with antimicrobial additives are common approaches to control *L. monocytogenes* post-processing contamination (Patterson et al., 1993; Fu et al., 1995; Gursel and Gurakan, 1997; Thayer and Boyd, 2000; Bedie et al., 2001; Muriana et al., 2002; Samelis et al., 2002; McCormick et al., 2003; Foong et al., 2004).

3.1. **In-package thermal pasteurization**

The effect of various surface pasteurization temperatures on the survival of *L. monocytogenes* were determined in low-fat turkey bologna (McCormick et al., 2003). At an 85 °C water bath temperature, no *L. monocytogenes* cells were detected in bologna after 10 s of exposure, whereas at 61 °C viable cells were detected up to 10 min of heating. The D-values for *L. monocytogenes* at 61 and 65 °C were 124 and 16.2 s, respectively. Z-values for *L. monocytogenes* was 4.44 °C, which indicates the in-package thermal pasteurization process, is effective in reducing or eliminating the contaminated *L. monocytogenes* cells (McCormick et al., 2003). Muriana et al. (2002) reported that heating at 90.6 to 96.1°C ≥2 min can readily provide 2-log reductions in most RTE deli meats. The effectiveness of in-package pasteurization in inactivating pathogenic organisms depends on package size. For vacuum packaged chicken fillets and strips artificially contaminated with a cocktail of *L. monocytogenes* culture, to achieve a 7-log10 (CFU/g) reduction, approximately 5, 25, and 35 min were needed for single-packaged fillets, 227-g package strips, and 454-g strips, respectively (Murphy et al., 2003a). The effectiveness of in-package surface pasteurization
was also affected by product surface roughness (Muriana et al., 2002; Murphy et al., 2003b).

The effectiveness of thermal processing depends on the *L. monocytogenes* strains. The thermal resistance of *L. monocytogenes* strains was studied by Lemaire et al. (1989). In an open vessel the D60 °C values ranged from 1.3-6.5 min, while in capillary tubes, D72 °C varied from 0.06-1.5 s (38 strains). Addition of 4.8% sodium lactate (SL) to beef increased heat resistance of *L. monocytogenes*. The D-values observed for beef with no SL or sodium diacetate (SDA) at 60, 65, 71.1, and 73.9 °C were 4.67, 0.72, 0.17, and 0.04 min, while with 4.8% SL had D-values ranging from 14.3 min at 60 °C to 0.13 min at 73.9 °C. SDA interacted with SL, thereby reducing the protective effect of SL and rendering *L. monocytogenes* in beef less resistant to heat (Juneja, 2003). Addition of 1.5 M-NaCl to *L. monocytogenes* cells grown at lower NaCl concentrations significantly increases the tolerance of cells to mild heat stress (56-62 °C) (Anderson et al., 1991). The factors such as growth and heating temperatures, type of plating medium, recovery method, and cell morphology have shown to influence the thermoresistance of suspended Listeria cells (P < 0.001) (Rowan and Anderson, 1998). Cells grown at 42.8 °C prior to heat treatment were more thermotolerance than that grown at 37 °C (Rowan and Anderson, 1998). Starvation increases the heat resistance of *L. monocytogenes* in broth but not in hot dog batter (Mazzotta and Gombas, 2001).

Heat shock and the type of media used to determine bacterial numbers during heat processing can significantly affect the D-values obtained (Walsh et al., 2001). They found that heat-shock did not significantly increase the D-values of wild type or antibiotic resistant strains in minced beef, while in potato slices D-values in almost all cases were significantly higher in samples which had received heat-shock treatments (Walsh et al., 2001). *L. monocytogenes* cells heat-shocked at 48 °C for 30 or 60 min did not show a significant increase in thermotolerance as compared with control cells (non-heat shocked), but bacteria heat shocked for 120 min did show an average 2.4-fold increase in D64 °C value (Farber and Brown, 1990). Heating at slowly rising temperatures is suspected to enhance thermotolerance in *L. monocytogenes* (Stephens et al., 1994; Hansen and Knochel, 1996). The increased
thermotolerance during slow rates of heating was analogous to the induction of heat-shock response (Stephens et al., 1994).

Listeria innocua M1 was developed as a thermal processing indicator organism for *L. monocytogenes* by selection of a rifampin- and streptomycin-resistant mutant. D (68 °C) values for *L. monocytogenes* and *L. innocua* were 3.8 and 4.9 s, respectively, in skim milk. The advantages of easy selection, similar heat resistance, and nonpathogenicity make *L. innocua* M1 appropriate for challenge studies designed to evaluate process lethality with respect to *L. monocytogenes* (Fairchild and Foegeding, 1993).

### 3.2. Irradiation

Ionizing irradiation is a process in which products are exposed to radiant energy. Irradiation has been successfully used to reduce pathogenic bacteria, eliminate parasites, decrease postharvest sprouting, and extend the shelf life of fresh perishable foods (Andrews et al., 1998). Irradiation includes gamma rays, electron beams, and X-rays. Gamma irradiation uses high-energy gamma rays from Cobalt 60 or Cesium 137, which has a long half-life and high penetration power, thus can treat bulk foods on shipping pallets. Electron beam (E-beam) irradiation uses a stream of high-energy electrons, known as beta rays, which can only penetrate several centimeters. Thus, foods are treated in thin layers. X-irradiation has intermediate properties of above two irradiation methods, which penetrates foods shallower than that of gamma irradiation but much deeper than that of electron beams (Sadler et al., 2001).

Irradiation has been studied extensively and is now in use worldwide. Decontamination of food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process. Candidates of radiation decontamination are mainly poultry and red meats, egg products, fishery products, spices and other dry ingredients (Farkas, 1998).

### 3.2.1 Radiation sensitivity and radiation resistance
A significant amount of research has been conducted to determine the effect of gamma irradiation in reducing *L. monocytogenes* in meat products (Patterson et al., 1993; Clavero et al., 1994; Gursel and Gurakan, 1997; Thayer et al., 1998; Lopez-Gonzalez et al., 1999; Thayer and Boyd, 1999, 2000; Clardy et al., 2002; Niemira et al., 2002; Sommers et al., 2003a,b). Compared with gamma-irradiation, few studies were conducted on E-beam irradiation. It was reported that E-beam irradiation was more effective than gamma-ray irradiation in decreasing *Bacillus cereus* and *Escherichia coli O157:H7*, but they were similarly effective for *L. monocytogenes* (Miyahara, 2002). In cooked pork chops and hams inoculated with *L. monocytogenes*, a low dose (0.75 to 0.90 kGy) of irradiation reduced *L. monocytogenes* by more than 2 log (Fu et al., 1995). Shamsuzzaman et al. (1995) reported that the combination of heating and e-beam irradiation was very effective in eliminating inoculated *L. monocytogenes* in chicken breast meat. The product that received both irradiation (3 kGy) and sous-vide (cooking in package) treatment had a shelf-life of at least 8 weeks at 8 °C, whereas the unirradiated samples treated with sous-vide spoiled in 16 days (Shamsuzzaman et al., 1995). Foong et al. (2004) reported that the e-beam irradiation doses needed for a 3-log reduction of *L. monocytogenes* were 1.5 kGy for bologna, roast beef, and turkey with and without lactate, and 2.0 kGy for frankfurters and ham (Foong et al., 2004). Clardy et al. (2002) found that the D10-values for gamma irradiation of *L. monocytogenes* artificially contaminated to ham and cheese sandwiches at -40 °C, ranged from 0.71 to 0.81 kGy, indicating that a dose of 3.5 to 4.0 kGy was needed to achieve a 5-log reduction of *L. monocytogenes*.

Compared with Gram-negative pathogens, *L. monocytogenes* are less sensitive to irradiation (Tarjan, 1990; Thayer, 1995). The sensitivity of *L. monocytogenes* to irradiation is different for different *L. monocytogenes* strains. In ground pork, the D10-values of *L. monocytogenes* with e-beam irradiation ranged from 0.372 kGy to 0.638 kGy depending on strains inoculated to ground pork (Tarte et al., 1996). The resistance of *L. monocytogenes* to radiation depends on the physiological state of the strain used (Augustin, 1996). In general,
cells under stress show higher levels of resistance to irradiation (Verma and Singh, 2001). For example, starvation enhances the radiation resistance of pathogens (Mendonca et al., 2004). *L. monocytogenes* strain, Scott A, exhibited the highest radiation resistance after 8 days of starvation. Starved cells consistently exhibited higher irradiation D_{10}-values than controls (P < 0.05) in both saline and ground pork (Mendonca et al., 2004). The resistance of *L. monocytogenes* to radiation also depends on the substrate in which the organism grow (Augustin, 1996). The sensitivity of *L. monocytogenes* to gamma irradiation was determined in trypticase soy broth supplemented with yeast extract in the slurry of chicken breast meat and in raw ground beef. D_{10} values for *L. monocytogenes* in these different media were 0.364, 0.599, and 0.699 kGy, respectively. The specific *L. monocytogenes* strain tested in that study appeared to be the most sensitive to irradiation in TSB-YE and most resistant in fresh minced beef (Gursel and Gurakan, 1997). Thayer et al. (1998) reported that gamma-radiation D_{10} values for *L. monocytogenes* were significantly different between raw and cooked nuggets, which are 0.56 +/- 0.03 kGy and 0.69 +/- 0.03 kGy, respectively; however, they were not significantly different (P < or = 0.05) between raw and cooked ground turkey meat (Thayer et al., 1998).

Sommers et al. (2003a) reported that adding sodium diacetate (SDA) and potassium lactate (PL) increased the sensitivity of *L. monocytogenes* to gamma irradiation. The D_{10}-value was 0.56 kGy for bologna without SDA and PL, 0.53 kGy for bologna containing 0.07% SDA-1% PL, and 0.46 kGy for bologna containing 0.15% SDA-2% PL (Sommers et al., 2003a). Salt content in product also affects the effectiveness of irradiation in killing pathogenic organisms. Highly significant effects (p < 0.01) of water content, water activity and NaCl content on the survival of *Salmonella typhimurium* in irradiated mechanically deboned chicken meat and ground pork loin were observed (Thayer et al., 1995). Their results suggested that the survival of foodborne pathogens in irradiated meats with reduced water content or increased NaCl levels might be greater than expected (Thayer et al., 1995). The radiation resistance of *L. monocytogenes* that was surface-inoculated onto bologna slices was
not affected by dextrose concentration (Sommers and Fan, 2002), but citric acid added into frankfurter enhanced the lethality of ionizing radiation (Sommers et al., 2003b).

The presence of oxygen affects the efficiency of irradiation. Irradiation treatments were significantly more lethal under aerobic packaging than in either vacuum or modified atmosphere packaging conditions (Thayer and Boyd, 1999). The effects of irradiation in conjunction with packaging vary depending upon the kind of meat and poultry and the atmosphere composition in the package (Lee et al., 1996). For ground turkey that received more than 1.0 kGy irradiation, there was a concentration-dependent CO₂ inhibition of L. monocytogenes multiplication and/or recovery (Thayer and Boyd, 1999). One concern about modified atmosphere packaging is that pathogens may grow and/or produce toxins in irradiated meat or poultry packaged using modified atmospheres because of lacking in competing organisms. This is of even greater concern if spoilage is suppressed and does not provide the usual warning signals (Lee et al., 1996).

Temperature effects must be carefully considered as reduced irradiation temperatures result in fewer adverse changes in the sensorial properties of meat and poultry products. However, low temperature conditions require greater radiation doses be used to inactivate the foodborne pathogen (Thayer, 1995). E. coli O157:H7 had a significantly (P < 0.05) higher D₁₀ value when irradiated at -17 to -15 °C than when irradiated at 3 to 5 °C (Clavero et al., 1994; Lopez-Gonzalez et al., 1999). The gamma radiation resistance, D₁₀ value, of an inoculated L. monocytogenes (ATCC 49594) in frozen vegetables, increased significantly with decreasing temperature from -5°C to -20°C (Niemira et al., 2002). The irradiation dose rate is another factor. At low dose rates, microbial enzymes may have more time to repair damage to cells, resulting in higher D₁₀ values or higher resistance (Lopez-Gonzalez et al., 1999).

All of the above results indicated that the manufacturers must take into account factors present in their products that allow L. monocytogenes to better resist to irradiation. Generalizations about effects of irradiation may be misleading if the irradiation conditions
and commodities are not specified (Diehl, 1992).

3.2.2 Mechanisms associated with irradiation-induced cell death and recovery

Few studies were conducted on the mechanisms of irradiation-induced cell death. Radiation-induced cell death is probably mediated primarily through deposition of energy in single events, a few vital macromolecules, or targets the integrity of which is indispensable for proliferation (Alper, 1977). The genome DNA is customarily regarded as the main target of ionizing and non-ionizing radiation (Alper, 1977; Verma and Singh, 2001). Extensive DNA damage following ionizing irradiation causes cell death.

Another important mechanism that irradiation-induced cell death is associated with is the ionizing radiation-generated reactive oxygen species that results in oxidative damage to the cell membrane (Mishra, 2004). Radiolysis of water can produce a variety of reactive species (Thakur and Singh, 1994). Hydrogen peroxide is an important reactive oxygen species that is one of the by-products of water radiolysis after exposure to ionizing radiation. The reaction of hydrogen peroxide with transition metals imposes on cells an oxidative stress condition that can result in damage to cell components such as proteins, lipids and principally to DNA, leading to mutagenesis and cell death (Asad et al., 2004). Of the fully hydrated lipid systems studied, saturated diacyl-phosphatidylcholines were most sensitive to radiation damage compared to ester- and ether-linked phosphatidylethanolamines and ether-linked phosphatidylcholines (Cheng and Caffrey, 1996). The presence of oxygen almost always sensitizes cells to irradiation damage (Alper, 1977). Besides peroxidation of membrane, other factors are also associated with membrane damage caused by irradiation. Research on a thermophilic bacterium, *Thermus thermophilus* HB-8, indicated that following irradiation, this bacterium lost its ability to take up extracellular K⁺ in a dose-dependent manner. Since the membranes of *Thermus thermophilus* HB-8 does not contain unsaturated fatty acids, this membrane damage caused by irradiation is not lipid peroxidation-related (Suzuki, 1985). The damage in membrane structure interferes with the normal metabolism of cells, especially the
generation of energy, and inhibits cell growth and death (Alper, 1977).

The DNA repair in *E. coli* following irradiation has been intensively studied. The repair involves a universal stress response that is induced by irradiation and is operated via the RecA regulator of the SOS response (Diez and et al., 2000). Few studies were conducted on the membrane repair of cells following irradiation, which warrants further studies. Fluorescence and electron spin resonance (ESR) studies from gamma-irradiation of liposomal vesicles showed that radiation-mediated lipid damage was modified by the inclusion of structure-modulating agents (e.g., cholesterol) and antioxidants (e.g., tocopherol, eugenol). The magnitude of modification of damage was found to be dependent on the concentration of these modifiers (Mishra, 2004). Moreover, experiments on dipalmitoyl phosphatidyl choline unilamellar liposomes demonstrated a biphasic behavior of radiation damage, which was remarkably modified by ascorbic acid and alpha-tocopherol in a concentration-dependent fashion (Mishra, 2004).

Overall, ionizing radiation can be an effective step in a HACCP program to kill enteric pathogens associated with meat and poultry products. The populations of most common enteric pathogens such as *Campylobacter jejuni*, *Escherichia coli O157:H7*, *Staphylococcus aureus*, *Salmonella spp.*, *L. monocytogenes*, and *Aeromonas hydrophila* can be significantly decreased or eliminated by low-dose (< 3.0 kGy) irradiation. Only enteric viruses and endospores of genera *Clostridium* and *Bacillus* are highly resistant to ionizing radiation, but even these are affected to some degree (Thayer, 1995).

### 3.3 High pressure processing

High pressure processing (HPP) is a novel nonthermal method of food processing where food is subjected to elevated pressures with or without addition of heat, which can inactivate microorganisms without significant changes in texture, color or nutritional value of food (Hugas et al., 2002; Ross et al., 2003). HPP is effective in reducing or eliminating most vegetative forms of bacteria at 300-800 MPa range (Solomon and Hoover, 2004). HPP is not only a powerful tool to control risks associated with pathogenic organisms such as *L.*
monocytogenes, Salmonella spp., and Campylobacter jejuni (Ananth et al., 1998; Ponce et al., 1998; Lucore et al., 2000; Tay et al., 2003; Sherry et al., 2004; Solomon and Hoover, 2004), it is also effective to spores and viruses (Kingsley et al., 2002; Reddy et al., 2003).

The resistance of microorganisms to HPP is variable depending on the types and strains of microorganism. Generally, Gram-positive organisms are more recalcitrant to pressure inactivation than Gram-negative bacteria (Hugas et al., 2002; Solomon and Hoover, 2004). Mussa et al. (1999) reported that L. monocytogenes inoculated to packaged fresh pork was more resistant to pressure inactivation than indigenous microflora in fresh pork (Mussa et al., 1999). Among nine tested L. monocytogenes strains, significant variability was observed in response to HPP (Tay et al., 2003). L. monocytogenes OSY-8578 exhibited the greatest pressure resistance, Scott A showed the greatest pressure sensitivity, and L. innocua had intermediate resistance (Tay et al., 2003). The decontamination efficacy of HPP also depends on many other factors such as level of pressure, treatment temperature, exposure time, pH, water activity and food composition (Hugas et al., 2002). Microbial inactivation was increased with prolonged exposure to pressure or increased pressure (Simpson and Gilmour, 1997; Ponce et al., 1998; Lucore et al., 2000). Pressurization at 700 MPa showed quick inactivation of L. monocytogenes (Lucore et al., 2000). HPP with 250 MPa did not inactivate L. monocytogenes, but significant lag phases of 17 and 10 days were observed at 5 and 10 °C, respectively (Lakshmanan and Dalgaard, 2004). Temperature influences the effectiveness of microbial inactivation by HPP. Inactivation of pathogens is less effective at optimal growth temperatures than inactivation at higher or lower temperatures of growth (Hugas et al., 2002). At 400 MPa, D values for Listeria innocua, a model microorganism for L. monocytogenes, was 7.35 min at 2 °C and 8.23 min at 20 °C (Ponce et al., 1998). In another study, Ananth et al. (1998) also showed that the effectiveness of HPP was slightly reduced at room temperature compared to refrigerated temperature (Ananth et al., 1998). The presence of oil reduces the effectiveness of high pressure in killing L. monocytogenes (Simpson and Gilmour, 1997). Cell morphology also has an effect on HPP, with bacilli being more sensitive to
pressurization than cocci (Hugas et al., 2002). When HPP was combined with antimicrobials, like bacteriocins, the death rate increased because of sub-lethal injuries to living cells (Garriga et al., 2002).

3.4 Food preservatives

3.4.1 Chemical antimicrobials

There are considerable reports about antimicrobial activities of salts of organic acids such as lactate, acetate and diacetate in meat products. Lactate is the most frequently used antimicrobials in meat products due to its beneficial properties to meat quality when applied at appropriate concentrations. Lactate addition to food products with a pH near neutrality offers good prospects for shelf life prolongation (Houtsma et al., 1993). In general, Gram-positive bacteria were more sensitive towards lactate than Gram-negative bacteria under optimum growth conditions (pH 6.5, 20 °C). It was shown especially that strains that were able to grow at water activities of 0.95 or lower in the presence of NaCl (Staphylococcus aureus, L. monocytogenes, Brochothrix thermosphacta) were inhibited by SL (Houtsma et al., 1993). Recently, more attention was paid to the combination application of lactate and diacetate due to the synergistic inhibitory effect of lactate and diacetate in inhibiting the growth of pathogenic organism (Bedie et al., 2001; Glass et al., 2002; Mbandi and Shelef, 2002; Samelis et al., 2002; Stekelenburg, 2003).

Qvist et al. (1994) suggested that it is possible to suppress growth of L. monocytogenes in chilled cooked meat products by using suitable amounts of SL combined with low pH conditions. The growth of L. monocytogenes was effectively suppressed up to 4 weeks in artificially contaminated vacuum packaged sliced bologna-type sausages formulated with 2% SL. No growth occurred in samples formulated with 2% SL and 0.25% glucono-delta-lactone during 35 days of storage at 5 °C and 10 °C (Qvist et al., 1994). Porto et al. (2002) reported that the addition of 2.0% (P < 0.0004) or 3.0% (P < 0.0001) potassium lactate as an ingredient in frankfurters can significantly enhance safety by inhibiting or delaying the
growth of *L. monocytogenes* during storage at refrigeration or abused temperature conditions. A mixture of 2.5% lactate and 0.25% acetate inhibited the growth of *L. monocytogenes* in vacuum-packed sliced RTE ham for 5 weeks at 4 °C (Blom et al., 1997). In turkey slurries, Schlyter et al. (1993) found that 2.5% lactate failed to suppress the growth of *L. monocytogenes* at 4 °C. Adding 2.5% lactate and 0.1% SDA combination prevented the growth of *L. monocytogenes* about 42 days at 4 °C, but 0.1% SDA itself was not effective, though 0.3% and 0.5% SDA in turkey slurries were listericidal at 4 and 25 °C, respectively (Schlyter et al., 1993). Samelis et al. (2002) reported that SL (1.8%; 3% of a 60% commercial solution) used in combination with 0.25% sodium acetate, SDA, or glucono-delta-lactone, inhibited the growth of *L. monocytogenes* in frankfurters for 120 days at 4°C. On cured smoked wieners, adding ≥1% SL plus ≥ 0.1% SDA inhibited the growth of *L. monocytogenes* for 60 days at 4.5 °C (Glass et al., 2002). Frankfurters formulated with 6% SL (60% commercial SL) or 0.5% SDA were bacteriostatic or even bactericidal throughout storage (120 days) at 4 °C. Adding 3% SL to frankfurters prevented *L. monocytogenes* growth for at least 70 days, while in decreasing order of effectiveness, SDA at 0.25% and sodium acetate at 0.5% and 0.25% (Bedie et al., 2001). Mbandi and Shelef (2001) reported that combinations of 2.5% SL and 0.2% SDA were bacteriostatic to *L. monocytogenes* in sterile comminuted beef for 20 days at 10 °C. At 5 °C, a listeriostatic effect was produced by 1.8% SL plus 0.1% SDA (Mbandi and Shelef, 2001).

In addition to direct addition during formulation, surface treatment with antimicrobials is also an effective way to reduce *L. monocytogenes* contamination. Beside lactic acid and SDA, many other chemical preservatives such as sodium benzoate, sodium propionate, potassium sorbate are used in surface treatment (Islam et al., 2002). Frankfurters were treated prior to inoculation by dipping or spraying onto their surface for 1 min in a solution containing one of four preservatives (sodium benzoate, sodium propionate, potassium sorbate, and SDA) at three different concentrations (15, 20, and 25%); the initial populations of *L. monocytogenes* at all three concentrations of all four preservatives treated frankfurters
decreased immediately by 1 to 2 $\log_{10}$ CFU/g. After 14 days of storage at 4 °C, L. monocytogenes counts for all treated frankfurters were 3 to 4 $\log_{10}$ CFU/g less than those for the untreated frankfurters (Islam et al., 2002). Washing raw chicken wings with a solution containing 0.5% lactic acid and 0.05% sodium benzoate greatly reduced the populations of pathogenic and psychotrophic bacteria, thus enhanced safety and extended shelf life (Hwang and Beuchat, 1995). Dipping artificially contaminated sliced bologna into organic acid and their salts before vacuum package was evaluated to control the growth of L. monocytogenes (Samelis et al., 2001). No significant (P > 0.05) increase in L. monocytogenes populations occurred in bologna slices treated with 2.5 or 5% acetic acid, 5% SDA or 5% PB from day 0 to 120. Products treated with 5% potassium sorbate and 5% lactic acid were stored for 50 and 90 days, respectively, before a significant (P < 0.05) increase in L. monocytogenes was observed (Samelis et al., 2001). Fresh beef sprayed with 0.5% cetylpyridinium chloride and 0.1% potassium sorbate mix inhibited L. monocytogenes growth during 14 days storage at 4 °C (Lim and Mustapha, 2004). Kolsarici and Candogan (1995) reported that 5% potassium sorbate was more effective than 3% lactic acid in inhibiting the growth of bacteria in vacuum-packaged raw chicken leg and breast meat (Kolsarici and Candogan, 1995).

The combination of food-packaging materials with antimicrobial compounds is a new type of active packaging to control surface contamination of microorganisms in foods (Vermeiren et al., 2002). Their potential food applications include vacuum or skin-tight packaged products, e.g. vacuum-packaged meat, where meat products are intensively contacted with packaging material. However, the 1000 mg/kg containing triclosan film did not effectively reduce spoilage bacteria and the growth of L. monocytogenes in refrigerated vacuum-packaged chicken breasts stored at 7 °C (Vermeiren et al., 2002).

Further, a chemically synthesized short-chain peptide composed of six leucine and eight lysine residues was demonstrated to be biocidal against several foodborne organisms including E. coli O157:H7, L. monocytogenes, Pseudomonas fluorescens, and Kluyveromyces marxianus suspended in phosphate buffer at concentrations of 5 to 50 µg/ml
(Appendini and Hotchkiss, 2000). Peptide concentrations of 100 \( \mu g/ml \) inhibited aerobic and anaerobic microorganisms present in meat exudate (Appendini and Hotchkiss, 2000). Trisodium phosphate was effective against \textit{L. monocytogenes} in chicken meat, especially after several days of refrigerated storage (Capita et al., 2001). Sodium hypochlorite, quaternary ammonium compound, and peroxyacetic acid are used as sanitizers in meat processing plant were effective in eliminating \textit{L. monocytogenes} (Romanova et al., 2002; Stopforth et al., 2002).

3.4.2 \textit{Lactobacilli, probiotic bacteria and bacteriocins}

Biopreservation with lactic acid bacteria is a suitable alternative to chemical preservatives (Jacobsen et al., 2003). Numerous strains of lactic acid bacteria were tested to be effective against \textit{L. monocytogenes} (Vignolo et al., 1993; Buncic et al., 1997; De Martinis and Franco, 1998; Bredholt et al., 2001; Amezquita and Brashears, 2002; Budde et al., 2003; Jacobsen et al., 2003). \textit{Lactobacillus casei} CRL 705 excreted substance, Lactocin 705, which was active against \textit{Lact. plantarum, L. monocytogenes, Staphylococcus aureus} (Vignolo et al., 1993). The antimicrobial activity of a bacteriocin-producing \textit{Lactobacillus plantarum} MCS strain against \textit{L. monocytogenes} was also observed in naturally and artificially contaminated salami (Campanini et al., 1993). The \textit{Lactobacillus sakei} strain applied to cooked products at a concentration of \( 10^5-10^6 \) CFU/g immediately before slicing and vacuum-packaging inhibited growth of \( 10^3 \) CFU/g of a cocktail of three rifampicin-resistant mutant \textit{L. monocytogenes} strains both at 8 °C and 4 °C (Bredholt et al., 2001). A combined culture of lactic acid strains - \textit{Pediococcus acidilactici, Lactobacillus casei, and Lactobacillus paracasei} - added to frankfurters and cooked ham coinoculated with \textit{L. monocytogenes} were vacuum packaged and stored at 5 °C for 28 days. Bacteriostatic activity was observed in cooked ham, whereas bactericidal activity was observed in frankfurters (Amezquita and Brashears, 2002). Jacobsen et al. (2003) reported that the live cells of bacteriocin producing \textit{Leuconostoc carnosum 4010} inhibited the growth of \textit{L. monocytogenes} in cooked, sliced and
gas packed meat products stored at 5 and 10 °C for 4 weeks (Jacobsen et al., 2003). This strain showed a strong antilisterial activity without producing any undesirable flavour components in meat products (Budde et al., 2003). Further, *Pediococcus, Bifidobacteria* and *Enterococcus* all showed strong antimicrobial activities towards *L. monocytogenes* (Mattila-Sandholm et al., 1991; Luchansky et al., 1992; Baccus-Taylor et al., 1993; Bevilacqua et al., 2003; Leroy et al., 2003; Mahoney and Henriksson, 2003; Sabia et al., 2003). The application of *lactobacillus, pediococcus* and *enterococi* bacteria in starter cultures may provide an additional hurdle against listeriosis in fermentation meat products (Luchansky et al., 1992; Baccus-Taylor et al., 1993; Hugas et al., 2003; Mahoney and Henriksson, 2003).

The main reason for the effectiveness of protective bacteria in killing *L. monocytogenes* is due to the production of bacteriocin (Baccus-Taylor et al., 1993; Campanini et al., 1993; Vignolo et al., 1993; De Martinis and Franco, 1998; Katla et al., 2002; Budde et al., 2003; Jacobsen et al., 2003). The production of organic acids by protective bacteria provides an additional antilisterial effect (Baccus-Taylor et al., 1993; Juven et al., 1998; Amezquita and Brashears, 2002; Katla et al., 2002). Katla et al. (2002) reported that both Sakacin P producing *Lactobacillus sakei* strain or no sakacin P producing *Lactobacillus sakei* strain had an inhibiting effect on the growth of *L. monocytogenes*, which indicates that factors other than bacteriocin sakacin P are active in inhibiting the growth of *L. monocytogenes* (Katla et al., 2002).

Bacteriocins are ribosomally-synthesized polypeptides produced by bacteria with an ability to kill or inhibit the growth of similar bacterial strain(s). Up to now, numerous bacteriocins have been characterized. Nisin is the most commercially important bacteriocin due to its relatively long history of safe use (Chen and Hoover, 2003). It is currently recognized as a safe food preservative in approximately 50 countries (Delves-Broughton et al., 1996). Nisin had an inhibitory effect on gram-positive bacteria such as *L. monocytogenes*, *Staphylococcus aureus*, and *Streptococcus lactis* though did not have any inhibitory effects
on gram-negative bacteria (Chung et al., 1989). Considerable research has been carried out on the antilisterial properties of nisin in foods. Beef cubes (2.5 x 2.5 x 2.5 cm) inoculated with approximately 7 log CFU/ml of *L. monocytogenes* Scott A and treated with nisin or nisin combined with EDTA by dipping in these solution for 10 min reduced the population of *L. monocytogenes* by 2.01 and 0.99 log CFU/cm² as compared to the control, respectively, under vacuum and storage at 4 °C for up to 30 days (Zhang and Mustapha, 1999). Four hundred IU/ml of nisin alone or combination of 400 IU/ml of nisin with 2% low-molecular-weight polylactic acid or 2% lactic acid showed immediate bactericidal effects on *L. monocytogenes* Scott A in vacuum-packaged beef (1.64-, 1.57- and 1.94- log10 reduction, respectively, compared with the initial number of 5.33 log10 CFU/cm² of the untreated control at day 0) (Ariyapitipun et al., 2000). Nisin incorporated into packaging materials also showed antilisterial activity. Lauric acid (8%, wt/wt) and 2.5% pure nisin (4%, wt/wt) were incorporated singly and together into thermally compacted soy films. Refrigerated bologna exposed to control films increased the cell number by 0.5 log from 10⁶ after 21 d at 4 °C. Nisin films reduced the cell number on turkey bologna from 10⁶ to 10⁵ after 21 d as did films containing nisin and lauric acid (Dawson et al., 2002). Packaging films coated with a cellulose-based solution containing 10,000 and 7,500 IU/ml nisin significantly decreased (P < 0.05) *L. monocytogenes* populations on the surface of hot dogs by > 2 log CFU per package throughout the 60-d study (Franklin et al., 2004).

Many bacteriocins other than nisin showed antilisterial activity. Reuterin produced by *Lactobacillus reuteri* strain 12002 at a concentration of 250 activity unit/g resulted in 3.0-log10 reduction of *L. monocytogenes* in raw ground pork after 1 week of storage at 7 °C (El-Ziney et al., 1999). The addition of purified sakacin P, bacteriocin produced by *Lactobacillus sakei*, to chicken cold cuts had an inhibiting effect on the growth of *L. monocytogenes*. A high dosage of sakacin P (3.5 μg/g) had a bacteriostatic effect throughout the storage period of 4 weeks, while a low dosage (12 ng/g) permitted initial growth, but at a slower rate. After 4 weeks of storage, the number of *L. monocytogenes* in the samples with a
low dosage of sakacin P was 2-log lower than that in the control (Katla et al., 2002). Enterocins, bacteriocins produced by enterococci, also hold considerable promise as alternatives to traditional chemical preservatives to control pathogens in meat products (Hugas et al., 2003; Leroy et al., 2003), and so was pediocin, a bacteriocin produced by *Pediococcus* (Foegeding et al., 1992; Luchansky et al., 1992; Baccus-Taylor et al., 1993; Degnan et al., 1993; Garriga et al., 2002). Both non-pediocin-producing and pediocin-producing starter cultures produced equivalent amounts of acid during fermentation, but pediocin-producing *Lactobacillus* starter culture showed greater *L. monocytogenes* inhibiting effect than the non-pediocin-producing starter cultures (Luchansky et al., 1992; Baccus-Taylor et al., 1993).

Current regulations, however, are hampering the application of purified bacteriocins (Hugas et al., 2003). Furthermore, bacteriocins are amphiphilic peptides susceptible to adsorption to food macromolecules and proteolytic degradation. These properties may limit their use as preservation agents (Aasen et al., 2003). Aasen et al. (2003) found that more than 80% of the added sakacin P and nisin were quickly adsorbed to proteins in food matrix. In foods that had not been heat-treated, proteolytic activity caused a rapid degradation of bacteriocins, with less than 1% of the total activity left after 1 week in cold-smoked salmon, and even less in raw chicken. In heat-treated foods, bacteriocin activity was stable for more than 4 weeks (Aasen et al., 2003).

### 3.4.3 Plant extract

Spices have a great potential to be used as antimicrobial agents. Garlic extract has a broad-spectrum of antimicrobial activity against many genera of bacteria and fungi (Adetumbi and Lau, 1983). Rosemary extract (Oxy'less) ethanol solution (100 mg/ml) has antibacterial activity to many pathogenic bacteria (Del Campo et al., 2000). The minimum concentration for inhibiting *L. monocytogenes* is 0.5%. Antibacterial activity of the rosemary extract was strongly influenced by the composition of media. Antibacterial activity is
increased by low pH, high NaCl contents, and low temperatures. Lipids, surface-active agents, and some proteins decreased its antibacterial activity. However, the inhibitory effect was little modified by heat treatment (100 °C). The antibacterial activity was linked to the compounds extracted with hexane, which are presumably phenolic diterpenoids (Del Campo et al., 2000).

Many other plant extracts have shown antibacterial activity against pathogenic bacteria. Essential oil of *Picea excelsa* has antilisteria activity, which was affected by several environmental factors. Introduction of sodium caseinate, agar or fat into the test medium and use of a cheese medium decreased the bactericidal effects of essential oils. Basic pH, addition of NaCl or use of Tryptone Soy Broth and saline solution increased its antilisterial activity (Canillac and Mourey, 2004). The essential oil from aerial parts of *Thymus eigii* was found to possess a strong antimicrobial activity (Tepe et al., 2004). The extract from petals of *Camellia japonica L.*, a member of the tea family, was also found to have antimicrobial activity (Kim et al., 2001).

There are also reports that addressed the effects of these compounds against pathogens associated with meat products. Hao et al. (1998) reported that eugenol (clove extract) and pimento extract significantly inhibited the growth of *A. hydrophila* and *L. monocytogenes* inoculated in cooked beef slices. However, *L. monocytogenes* was not as sensitive as *A. hydrophila* to both treatments, especially to pimento extracts (Hao et al., 1998). Cutter (2000) suggested that the use of herb extracts reduced pathogens on beef surfaces; however, the antimicrobial activity might be diminished in ground beef by adipose components. Larson et al. (1996) reported that hop extracts could be used to control *L. monocytogenes* in minimally processed food with low fat content. The numbers of *E. coli O157:H7*, *L. monocytogenes*, and *Salmonella Typhimurium* declined by 1.08, 1.24, and 1.33 log CFU/g, respectively, in raw ground beef treated with 1% pine bark extract (Pycnogenol) after 9 days of refrigerated storage (Ahn et al., 2004). Grape seed extract ActiVin (1%) and rosemary oleoresin (1%) resulted in an approximately 1-log CFU/g reduction in the populations of all three pathogens
after 9 days. The results suggest that these natural extracts have potential to be used with other preservation methods to reduce pathogen numbers in ground beef (Ahn et al., 2004).

3.5 Hurdle technology

The concept of hurdle technology was first proposed by Dr. Lothar Leistner at the Federal Centre for Meat Research, Kulmbach, Germany (Leistner, 1978). This technology is based on the application of combined preservative factors to achieve microbiological safety and stability of foods (Leistner, 1978). The most important hurdles used in food preservation are temperature, water activity, acidity, redox potential, antimicrobials, and competitive microorganisms (Leistner, 2000). A synergistic effect could be achieved if the hurdles in a food hit at the same time, different targets that disturb the homeostasis of the microorganisms present in foods (Leistner, 2000).

For RTE meat products, the most frequently applied hurdles include thermal processing, vacuum packaging, refrigerated storage and nitrite. But these hurdles seem insufficient when it comes to *L. monocytogenes* due to its ubiquitous nature (Beresford et al., 2001), its ability to grow at refrigerated temperature and anaerobic condition, and its resistance to salt and nitrite (Lou and Yousef, 1999). Post processing contamination of RTE meat with *L. monocytogenes* during slicing and packaging is difficult to avoid, thus, to ensure microbiological safety of RTE meats, apparently additional hurdles are needed to control growth of the pathogen during product storage. Formulating meat products with antimicrobial additives are common practice to control *L. monocytogenes* post-processing contamination (Bedie et al., 2001; Glass et al., 2002; Mbandi and Shelef, 2002; Samelis et al., 2002; Stekelenburg, 2003). The post-package decontamination such as postpackage pasteurization (Muriana et al., 2002), irradiation (Patterson et al., 1993; Fu et al., 1995; Gursel and Gurakan, 1997; Thayer and Boyd, 2000; Foong et al., 2004) and HPP (Ananth et al., 1998; Lucore et al., 2000; Tay et al., 2003) are additional hurdles for RTE meats.

Irradiation is very effective in eliminating contaminated *L. monocytogenes*. However,
pathogens survived irradiation could grow and proliferate during refrigerated storage (Foong et al., 2004), and thus additional hurdles are needed. Antimicrobials were used in combination with irradiation to suppress the growth of \textit{L. monocytogenes} following irradiation. Irradiation of \textit{L. monocytogenes} suspended in SDA resulted in synergistic reductions of the microorganism. SDA can inhibit the proliferation of \textit{L. monocytogenes} surviving irradiation process when used within regulatory limits (Sommers and Fan, 2003). An ionizing radiation dose of 3.0 kGy prevented the proliferation of \textit{L. monocytogenes} and background microflora in bologna containing 0.07% SDA and 1% potassium lactate, and in bologna containing 0.15% SDA and 2% potassium lactate over 8 weeks of storage at 9 °C (Sommers et al., 2003a). Adding bacteriocins such as nisin and pediocin Ach into formulation is another choice. Including bacteriocins into meat formulation increased pathogen death rate during high pressure processing (Garriga et al., 2002).

Part II. Irradiation and Preservatives on the Quality of Meat Products

1. Irradiation and meat quality

1.1 Irradiation odor

Irradiation has been showed to be very effective in controlling food-borne pathogens (Farkas, 1998). However, ionizing radiation generates free radicals that cause lipid peroxidation and other chemical changes, and influence the quality of foods (Hashim et al., 1995a, b; Patterson and Stevenson, 1995; Ahn et al., 1998a, b; Ahn and Lee, 2004). Irradiated meat products can develop a characteristic odor described as "barbecued corn-like" or "bloody sweet" (Ahn and Lee, 2004). Hashim et al. (1995b) reported that raw irradiated chicken had higher "fresh chickeny, bloody, and sweet aromatic aroma" intensities compared to nonirradiated samples. Patterson and Stevenson (1995) isolated volatiles from irradiated raw chicken by gas chromatography in conjunction with olfactometry assessment and found
that dimethyl trisulphide was the most potent and obnoxious compound, followed by *cis*-3, *trans*-6 nonenals, 1-octen-one and bis (methylthio-) methane. More researches indicated that the volatiles responsible for the off-odor in irradiated meat are sulfur-containing compounds such as methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide (Ahn et al., 2000a, b; Fan et al., 2002; Nam and Ahn, 2003a). All these sulfur compounds were generated by the radiolytic degradations of sulfur-containing amino acids present in meat (Ahn et al., 2000a, b; Ahn and Lee, 2004). Ahn et al. (2000a, b) reported that dimethyl disulfide and other sulfur compounds increased dramatically following irradiation. Fan et al. (2002) reported that the concentrations of hydrogen sulfide, sulfur dioxide, methanethiol, and dimethyl disulfide in pre-cooked RTE turkey breast meat dramatically increased following gamma irradiation. In addition to sulfur compounds, irradiation dramatically increases other volatiles in the headspace of meat products. The formation of volatiles during irradiation is associated with the radiolysis of meat components, mainly amino acids and fatty acids (Ahn et al., 2000a, b; Jo and Ahn, 2000).

1.2 Degradation of amino acids, fatty acids and other compounds by irradiation

Irradiation can radiolyze amino acids and generate many new volatiles (Jo and Ahn, 2000; Ahn, 2002). Each amino acid homopolymer group produced different odor characteristics, but the majority of newly generated and increased volatiles by irradiation were sulfur compounds that were produced by sulfur-containing amino acids during irradiation. The overall odor intensity of irradiated sulfur amino acids was very high and the odor characteristics of sulfur amino acids were similar to irradiation odor of meat (Ahn, 2002; Ahn and Lee, 2002). Irradiating leucine and isoleucine produces 3-methyl butanal and 2-methyl butanal, respectively. Dimethyl disulfide was formed when irradiating methionine, and carbon disulfide was formed when irradiating cysteine (Jo and Ahn, 2000). Ahn (2002) found that the contribution of methionine to the irradiation odor would be far greater than that of cysteine. Mechanisms related to the radiolysis of amino acids are not fully understood,
but deamidation during irradiation is one of the main steps involved in amino acid radiolysis (Dogbevi et al., 1999). The degradation of amino acids by oxidative deamination-decarboxylation via Strecker degradation produces branched chain aldehydes (Mottram et al., 2002), which may be the mechanism for the formation of 3-methyl butanal and 2-methyl butanal during irradiation. Davies (1996) reported that irradiation of N-acetyl amino acids and peptides in the presence of oxygen gives high yields of side-chain hydroperoxides, which can be formed on both the backbone (at alpha-carbon positions) and the side chain (Davies, 1996). Decomposition of alpha-carbon hydroperoxides by Fe(II)-EDTA gives initially an alkoxy radical, which may be the key intermediate in the fragmentation of proteins in the presence of oxygen. With N-acetyl amino acids and dipeptides beta-scission of an alkoxy radical at the C-terminal alpha-carbon results in C-terminal decarboxylation (Davies, 1996). More than one site in amino acid side chains was labile to free radical attack, and many volatiles were produced by the secondary chemical reactions after the primary radiolytic degradation of side chains (Ahn and Lee, 2002). Irradiation induces the formation of hydrogen and carbon monoxide (CO), which should be the by-product of radiolysis of amino acids and other compounds (Hitchcock, 1995; Nam and Ahn, 2002a, b).

Besides amino acids, fatty acids are also radiolyzed by irradiations. When triglycerides or fatty acids are irradiated, hydrocarbons are formed by cutting CO₂ and CH₃COOH off from fatty acids in various free-radical reactions (Morehouse et al., 1993). The yield of these radiolytically generated hydrocarbons was linear with absorbed dose (Morehouse et al., 1993; Miyahara et al., 2002). Radiolytic degradation of fatty acid methyl ethers were affected by irradiation dose, irradiation temperature, oxygen pressure, and fatty acid components (Miyahara et al., 2002). Polyunsaturated fatty acids (PUFA) are more susceptible to radiolysis than monounsaturated or saturated fatty acids and irradiation caused a significant reduction in PUFA (Formanek et al., 2003). It is hypothesized that the mechanism for the radiolysis of fatty acids is similar to lipid oxidation, especially when oxygen is available. Jo et al. (1999) showed that 1-heptene content in volatiles was positively correlated to
irradiation dose and Du et al. (2001b) showed that the production of alkenes and alkanes also increased proportionally to irradiation dose. All these compounds are the degradation products of fatty acids.

Irradiation causes some degradation of vitamins. Thiamin (vitamin B1) is the most radiation sensitive among the water-soluble vitamins (Graham et al., 1998). Irradiation induces a slight reduction in thiamine levels in chicken meant (Shamsuzzaman et al., 1992; Graham et al., 1998). Fox et al. (1995) reported that thiamin loss was an average of 11%/kGy while riboflavin loss was 2.5%/kGy above 3.0 kGy. The rate of thiamin loss in beef was higher than that in lamb, pork and turkey leg with losses of 16%/kGy in beef and 8%/kGy in lamb. The incurred losses were unlikely to be of nutritional significance (Graham et al., 1998; Lakritz et al., 1998). The rate of thiamin loss was not related to sulfhydryl, protein, moisture, fat or water content, pH or reducing capacity (Fox et al., 1995). The loss of thiamine increased with the increase of irradiation doses and temperatures (Lee and Hau, 1996).

Bagorogoza et al. (2001) reported that irradiation at 2.4 to 2.9 kGy reduced alpha-tocopherol levels by 33% in turkey breast meat. Lee et al. (1993) found that irradiation significantly decreased vitamin A level in irradiated Cheddar cheese and mozzarella cheese. Ahn et al. (2002) found that gamma-irradiation could significantly (P < 0.05) reduce residual nitrite in meat products. Following 4 weeks of storage, N-nitrosodimethylamine and n-nitrosopyrrolidine levels were significantly decreased in the vacuum packaged sausages irradiated at 20 kGy compared with sausage without irradiation (Ahn et al., 2002).

1.3 Irradiation and color

Irradiation causes a variety of color changes, including red, brown or green colors, which are related to the concentration of heme pigments and the status of heme iron, pH and reducing potential of meat, and temperature and packaging conditions during irradiation (Nam and Ahn, 2003b; Brewer, 2004). Usually light meat produces pink color while dark meat becomes brown or gray after irradiation (Nam and Ahn, 2003b). Irradiated turkey and
chicken breast meat had a higher a* value, showing an increase in redness in color (Du et al., 2001c; Lewis et al., 2002; Nam and Ahn, 2002a, b). But for irradiated raw beef, a* values decreased (Nanke et al., 1998; Nam and Ahn, 2003c; Ahn and Nam, 2004) and yellowness increased with dose and storage time (Nanke et al., 1998).

The color change induced by irradiation is associated with CO production during irradiation (Nam and Ahn, 2003b; Ahn and Lee, 2004). Nam and Ahn (2002a, b) showed that irradiation increased the production of CO, which is correlated with the increased redness of irradiated meat. The absorption spectra of carbon monoxide-myoglobin (CO-Mb) was very similar to that of the myoglobin extracted from irradiated meat, and thus it was concluded that CO production and CO-Mb complex formation was the main reason for the increased redness in irradiated turkey breast meats (Nam and Ahn, 2002a, b). The extent of color change was irradiation dose-dependent (Nanke et al., 1998). The oxidation-reduction potential of meat was lower in electron-beam irradiated meat than that of nonirradiated meat (Nam and Ahn, 2002a; Du et al., 2001c). The low oxidation-reduction potential plays an important role in the formation of CO-Mb. Irradiated turkey breast meat supplemented with vitamin E had a more intense pink color than control samples (Nam et al., 2003), which indirectly proved the contribution of a low redox potential on the redness induced by irradiation. The production of carbon monoxide and changes of oxidation-reduction potential in red meats by irradiation were similar to those of light meat, the different color changes were due to high pigment content in red meat (Nam and Ahn, 2003b).

1.4 Irradiation, water holding capacity and texture

Irradiation affects the water holding capacity of meat. Irradiated chicken breasts had more cooking loss than nonirradiated chicken breasts (Yoon, 2003). Zhu et al. (2004) found that irradiation significantly increased centrifugation loss of water from pork loins compared to that of non-irradiated samples (Zhu et al., 2004). The mechanism for irradiation-induced water loss is not clear, but two possible theories exist: 1) irradiation may damage the integrity
of membrane structure of muscle fibers (Lakritz et al., 1987), and 2) irradiation may denature muscle proteins, thus reducing water holding capacity (Lynch et al., 1991). Transmission electron microscopy showed significant differences (P < 0.0001) in size of myofibril units (sarcomeres) between irradiated and nonirradiated breasts. Shrinkage in sarcomere width (myofiber diameter) and disruption of myofibrils in irradiated breast meat were also noticed when compared with nonirradiated breast meat (Yoon, 2003).

Consumer taste panels indicated that, at day 0, there were no differences in texture among controls and irradiated (1.0 kGy and 1.8 kGy) chicken breasts, but the texture attributes were lower in irradiated samples after 14 d and 28 d storage at 0 °C (Lewis et al., 2002). Luchsinger et al. (1997) reported that irradiation had minimal effects on texture of frozen, raw and precooked, ground beef patties; frozen boneless beef steaks; and vacuum-packaged, chilled, boneless, beefsteaks. Yoon (2003) found that irradiated chicken breasts had higher (P < 0.0001) shear force than nonirradiated chicken breasts.

1.5 Irradiation and oxidation

1.5.1 Lipid oxidation and meat quality

Lipid oxidation is the primary cause of quality deterioration in cooked meats. Quality change associated with oxidation includes off-flavor or off-odor development, discoloration, adverse changes in texture, drip loss, loss of nutritional value, loss of functionality, and the generation and accumulation of compounds that may be detrimental to the health of consumers (Gray et al., 1996; Morrissey et al., 1998).

Lipid oxidation is comprised of three steps: initiation, propagation and termination. Lipid oxidation is initiated by the attack of any species that has sufficient reactivity to abstract a labile hydrogen atom from a methylene group in lipid molecules to form a lipid radical. PUFA is more susceptible to such an attack than fatty acids with one or no double bonds (Wagner et al., 1994). Phospholipids contain high level of unsaturated fatty acids, mainly linoleic and arachidonic acids, and thus tend to be oxidized easily (Ahn et al., 1998a).
Propagation is characterized by the ability of those free radicals to obtain hydrogen atoms from other fatty acids that result in the proliferation of oxidation. The quenching of free radicals terminates the oxidation process.

Lipid oxidation forms an oxidation off-flavor characterized as a cardboard, warmed-over, or rancid/painty flavor (Ang and Lyon, 1990). Malonaldehyde is one of the secondary oxidation byproducts of lipids. Thiobarbituric acid reactive substances (TBARs) measures malonaldehyde content in meat and its presence in meats is an indicator of lipid oxidation. Hexanal is also used as an indicator of lipid oxidation, which is well correlated with TBARs values (Chen et al., 1997). Both hexanal and TBARs are highly correlated with lipid oxidation in meat products, and correlated with the warmed-over flavor of meat (Lai et al., 1995; Du et al., 2001a). Other aldehydes, including propanal, pentanal and heptanal are also associated with TBARs values (Igene et al., 1985; Nolan et al., 1989; Su et al., 1991; Lai et al., 1995; Chen et al., 1997; Ahn et al., 1998a, b; 2000b). The overall off-flavor characteristics increased with the increase of TBARs and aldehydes content in headspace of the meat sample (Ang and Lyon, 1990).

Lipid oxidation and myoglobin oxidation are interactive with each other. The formation of metmyoglobin from oxymyoglobin is related to lipid oxidation and dependent on the antioxidant status (Gorelik and Kanner, 2001). Antioxidants retard the production of lipid oxidation products that catalyze the oxymyoglobin oxidation (Yin et al., 1993; Gray et al., 1996; Gorelik and Kanner, 2001). On the other hand, released non-haem iron is believed to be the primary catalyst accelerating lipid oxidation (Jayathilakan et al., 1997). In a model system, Conalbumin (50 μmol/L), a specific iron chelator, inhibited lipid peroxidation and oxymyoglobin oxidation by almost 50% (Gorelik and Kanner, 2001). Meat with elevated levels of vitamin E possesses greater oxymyoglobin and lipid stability, which results in less discoloration and rancidity, respectively (Yin et al., 1993; Schaefer et al., 1995; Gorelik and Kanner, 2001). In an oxymyoglobin and liposome model, the increase in unsaturated fatty acids increased lipid oxidation and oxidation of heme proteins (Yin and Faustman, 1993).
Packaging determines the availability of oxygen to meat, and thus, affects lipid oxidation during storage. Poultry meat patties packaged and stored under vacuum conditions had significantly lower lipid oxidation than those stored under aerobic conditions (Luchsinger et al., 1996; Ahn et al., 2000a, b; Du et al., 2000). The low oxygen transmission rate for the vacuum package bags is associated with a low off-odor formation (Dawson et al., 1995). Modified atmosphere packaging by decreasing oxygen also delays lipid oxidation (Nolan et al., 1989). To avoid lipid oxidation, vacuum packaging and antioxidants are frequently used (Formanek et al., 2003; Ahn et al., 2000a).

1.5.2 Irradiation and lipid oxidation

Ionizing radiation may cause a hydrogen atom removal from the unsaturated hydrocarbon of unsaturated fatty acids to form a free radical, and thus initiates oxidation. However, electron beam irradiation accelerated lipid oxidation in meat only under aerobic conditions (Du et al., 2001b; Ahn and Lee, 2004), and the types and amounts of volatiles produced by irradiation did not correlate well with the degrees of lipid oxidation (Ahn and Lee, 2004). Vacuum packaging is superior to aerobic packaging for irradiation and subsequent storage of meat because the vacuum package minimizes oxidative changes in meat (Du et al., 2001b; Ahn and Lee, 2004). Vacuum-packaged irradiated samples, however, retained sulfur volatile compounds (methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide) mainly responsible for the irradiation off-odor during storage (Ahn et al., 2000a; Nam and Ahn, 2003a). Sulfur compounds were highly volatile and could be eliminated by storing the irradiated meat under aerobic conditions (Ahn et al., 2000a; Ahn and Lee, 2004). These facts lead to the idea of double-packaging for meat irradiation (Nam and Ahn, 2003a; Ahn and Lee, 2004). That is, before irradiation, meat samples were first aerobic-packaged, then vacuum-packaged. Nam and Ahn (2003a) found that exposure of double-packaged irradiated turkey meats to aerobic conditions by removing outer vacuum bags a few days before sensory evaluation was effective in controlling both lipid
oxidation-dependent (aldehydes) and radiolytic off-odor (sulfur compounds) volatiles.

Irradiation also accelerates the oxidation of cholesterol. The amounts of cholesterol oxidation products were higher in irradiated cooked turkey, pork, and beef patties than those without irradiation (Du et al., 2001b; Nam et al., 2001). The amounts of cholesterol oxidation products and lipid oxidation products are closely related to the proportion of polyunsaturated fatty acids in meat (Ahn et al., 2001). Polyunsaturated fatty acid-enriched diets increased meat susceptibility to oxidation (Grau et al., 2001). Irradiation is also reported to cause the oxidation of amino acids by generating high yields of side-chain hydroperoxides that relates to the oxidation of proteins and lipids (Davies, 1996).

### 1.5.3 Antioxidants and lipid oxidation

alpha-Tocopherol, a natural lipophilic antioxidant, can quench lipid peroxyl and alkoxyl radicals by donating labile hydrogen. Due to delocalization of the unpaired electron into the aromatic ring structure, the resulting tocopheryl radical is insufficiently reactive to abstract the hydrogen atom from fatty acids, and thus stop the chain reaction of lipid oxidation (Fukuzawa and Gebicki, 1983). Supplementation of tocopherol is effective in preventing oxidative off-odor, such as fishy taints and warmed-over flavor in meat. Gatellier et al. (2000) reported that vitamin E supplementation significantly decreased radical production. Adding alpha-tocopherol alone or combination with other antioxidants to ground or minced beef before irradiation was effective in reducing lipid oxidation (Formanek et al., 2003; Ahn and Nam, 2004). Adding tocopherol to the diet, improved the oxidative stability of chickens and turkeys during freezing, thawing, and refrigerated storage (Bartov et al., 1983; De Winne and Dirinck, 1996; Sheldon et al., 1997; Grau et al., 2001). Govaris et al. (2004) reported that dietary supplementation of 200 mg alpha-tocopherol/kg to turkey retarded lipid oxidation in both breast and thigh meat patties at all storage times compared with controls. Ahn et al. (1997) reported that dietary alpha-tocopheryl acetate at > 200 IU/kg diet decreased lipid oxidation and reduced total volatiles of raw turkey patties. Dietary alpha-tocopheryl acetate
supplement at a level of more than 200 IU/kg in diet was also helpful in maintaining low
TBARs values in irradiated cooked turkey breast and leg meat patties during the 7-days
storage period (Ahn et al., 1998b). Galvin et al. (1998) reported that gamma-irradiation had
little effect on lipid stability in alpha-tocopherol-supplemented meat following cooking and
storage. In general, supplementation with or over 200 mg alpha-tocopheryl acetate/kg feed
can control lipid oxidation in chicken or turkey meat (Morrissey et al., 1997; Ahn et al.,
1998b; Galvin et al., 1998; Grau et al., 2001; Govaris et al., 2004). To control cholesterol
oxidation, supplementation with over 400 mg alpha-tocopheryl acetate/kg feed may be
required (Galvin et al., 1998).

Besides alpha-tocopherol, numerous other antioxidants are reported to be effective in
preventing oxidative off-odor. Ascorbate can regenerate the chain breaking antioxidant,
alpha-tocopherol, in biological systems (Kagan et al., 2003), thus, vitamin C and vitamin E
have cooperative effects in vivo. However, ascorbate supplementation itself appears to have
little, if any, beneficial effects on meat lipid stability (Grau et al., 2001; Morrissey et al., 1998,
King et al., 1995). Carnosine, a beta-alanyl-histidine dipeptide naturally occurs in skeletal
muscles, exhibits antioxidant properties in meat products. Inclusion of 2-10 mmol/L
carnosine in a meat-related model system (liposomes of muscle phospholipids) resulted in
decreased lipid oxidation (Kansci et al., 1997). The combination of carnosine (50 mmol/L)
with ascorbic acid (500 ppm) provided an excellent antioxidative protection with regard to
the oxidative deterioration of beef steak packaged in modified atmosphere (Djenane et al.,
2004). Post-slaughter carnosine addition may be an effective means of improving lipid
stability in processed meats (Morrissey et al., 1998). Han and Yamauchi (2000) indicated that
the addition of 100 ppm nitrite to ground pork resulted in a remarkable antioxidant effect
during refrigerated storage. The presence of ascorbic acid and/or nitrite seems important for
the protection of cholesterol oxidation in fermented meat products (Zanardi et al., 2004).
Jayathilakan et al. (1997) reported that clove and Maillard reaction products exhibited very
good antioxidative effects in mutton, pork and chicken.
Recently, much attention was paid to plant extracts due to their antioxidant and antimicrobial activity (Del Campo et al., 2000; Chidambara Murthy et al., 2003; Ahn et al., 2004; Tepe et al., 2004). Dietary supplementation of tea catechins to chickens at above 100 mg/kg feed exerted antioxidative effects in chicken breast (Tang et al., 2000). The antioxidant potential of tea catechins was two- to four-fold greater than that of α-tocopherol at the same concentration (Tang et al., 2001). Thomson and Ali (2003) reported that aged garlic extract exhibited radical scavenging activity. S-allylcysteine and S-allylmercapto-L-cysteine are two major compounds that had the highest radical scavenging activity. Ahn et al. (2004) reported that grape seed extract (ActiVin) and pine bark extract (Pycnogenol) effectively retarded the lipid oxidation in raw ground beef. Rosemary extract is a potential antioxidant in meat products (Del Campo et al., 2000; Du and Ahn, 2002; Formanek et al., 2003; Lee et al., 2003a). Sesamol was also effective in controlling lipid oxidation and off-odor intensity in irradiated meat products (Chen et al., 1997; Du and Ahn, 2002; Lee and Ahn, 2003). Lee et al. (2003b) reported that infrared-treated rice hull extracts showed significant antioxidant activities and was effective in reducing the production of dimethyl disulfide responsible for irradiation off-odor in irradiated raw and cooked turkey meat during aerobic storage (Lee et al., 2003b; Nam et al., 2004). The antioxidant activity of rice hull extracts (0.1%, wt/wt) was as effective as that of rosemary oleoresin (0.1%) (Lee et al., 2003b), but antioxidant effect of 0.2% rice hull extracts was lower than that of 0.01% sesamol (Nam et al., 2004).

2. Consume response to irradiation

2.1 Consume response to flavor and color associated with irradiated meat

One of the major problems for irradiation is its side-effect on the flavor of products. Hamburgers made with patties treated with 4.5 kGy were rated lower (P < 0.05) in taste than hamburgers made with either control patties or those treated with 3.0 kGy (6.5, 6.6, and 6.2, respectively, for 0, 3.0, and 4.5 kGy) (Wheeler et al., 1999). Irradiated raw pork *longissimus dorsi* muscle strips produced stronger irradiation odor than nonirradiated muscle, and
approximately 70% of sensory panels characterized irradiation odor as barbecued-corn-like odor (Ahn et al., 2000b). Du et al. (2003) showed that irradiation caused a metallic off-flavor in chicken rolls. Hashim et al. (1995b) found cooked irradiated frozen dark chicken meat had more chicken flavor. Al-Bachir and Mehio (2001) reported that luncheon meat exposed to gamma-irradiation up to 4 kGy had no significant differences (P > 0.05) in taste and flavor from that in non-irradiated samples.

Unlike flavor, consumers accepted the color of irradiated meats. Consumers linked the redness caused by irradiation to freshness and preferred the color of irradiated raw and cooked meats (Lee et al., 2003a). For uncured cooked poultry breast meat, however, the redness was linked to undercooked or contaminated products and negatively affected consumer acceptance (Nam and Ahn, 2002a, b).

2.2 Consumers acceptance of irradiation

Food irradiation, a new technology, has captured the interest of government, industry and consumers in recent years (Derr, 1993). A survey by FoodNet indicated that nearly one-half (49.8%) of the 10,780 adult respondents were willing to buy irradiated meat or poultry (Frenzen et al., 2001). The most important reasons why adults would not buy irradiated meat and poultry were insufficient information about risks and/or benefits, which accounted for 35%, and concerns about safety of eating irradiated food, which accounted for 22.7% (Frenzen et al., 2000). A simulated supermarket setting test was conducted to determine whether consumers (n = 126) would purchase irradiated poultry products (Hashim et al., 1995a). The result showed that about 84% of the participants considered it either "somewhat necessary" or "very necessary" to irradiate raw chicken and would like all chickens that was served in restaurants or fast food places to be irradiated. About 44% of the participants were willing to pay the same price for irradiated chicken as for nonirradiated. About 42% of participants were willing to pay 5% or more than what they were currently paying for nonirradiated chicken (Hashim et al., 1995a). In another survey, more than 64% of
the respondents reported that they preferred ground beef treated by irradiation to that treated by heat pasteurization (Fingerhut et al., 2001). Overall, consumers view irradiated meat and poultry products positively (Bruhn, 1995a). However, a report from a number of supermarket chains showed that the word “irradiation” was negative to consumers (Patricia, 2002).

The major concern of consumers about the irradiated foods was health risks from irradiation by-products. Consumers need to learn what irradiation is and why it is used to process foods. That information will better enable them to make informed decisions about irradiated foods. Numerous studies have demonstrated that acceptance increases when consumers are provided with information about specific advantages of the irradiation process (Bruhn, 1995b; Hashim et al., 1995a; Frenzen et al., 2000; Hayes et al., 2002). The number of respondents who are willing to purchase irradiated meat products increased significantly after an educational program, from 59.5 and 61.9% to 83.3 and 85.7% for the breasts and thighs, respectively (Hashim et al., 1995a). This is concurred by another report (Frenzen et al., 2000). A favorable description of irradiation increased willingness-to-pay, and an unfavorable description decreased willingness-to-pay. When subjects were given both pro- and anti-irradiation descriptions, the negative description dominated and willingness-to-pay decreased (Fox et al., 2002). Major studies in the United States indicated that the number of consumers concerned about the safety of irradiated food has decreased in the last 10 years and continues to be less than the number of those concerned about pesticide residues, microbiological contamination, and other food-related concerns (Bruhn, 1995a). Marketing of irradiated food in the United States, although limited, has been successful (Bruhn, 1995a, b).

3. Antimicrobials and meat quality

The antimicrobial activities of salts of organic acids such as lactate, acetate and diacetate in meat products are well documented (Blom et al., 1997, Bedie et al., 2001; Stekelenburg and Kant-Muermans, 2001; Glass et al., 2002; Mbandi and Shelef, 2002;
Commercial lactate is a natural salt that is derived from a bacterial fermentation product, lactic acid, which naturally produced in muscle tissues. The production of lactic acid is an important part of the energy metabolism of muscle tissues. SDA is a molecular mixture of acetic acid and sodium acetate. The US Food and Drug Administration (FDA) affirmed SDA as a direct food substance generally recognized as safe and permitted its use in food at level up to 0.25%. SL is used to improve microbial stability, enhance flavor, increase shelf-life and reduce moisture loss. The interest to lactate and SDA in the meat industry is growing, due to the synergistic inhibitory effect of lactate and diacetate combination in inhibiting the growth of pathogenic organisms (Bedie et al., 2001; Glass et al., 2002; Mbandi and Shelef, 2002; Samelis et al., 2002; Stekelenburg, 2003).

Papadopoulos et al. (1991) reported that injection of SL to cooked, vacuum-packaged beef top rounds resulted in higher cooking yields and darker, redder color with less gray surface area. Flavor notes associated with fresh beef were also enhanced by the addition of SL, and flavor deterioration during storage was minimized. In Chinese-style sausage, the addition of 3% SL resulted in better quality regarding physicochemical characteristics (Lin and Lin, 2002). Jensen et al. (2003) reported that lactate/diacetate-enhanced chops maintained higher a* and b* values during display and had less visual discoloration after 96 h display. Chops pumped with lactate, acetate or lactate/diacetate mixture were more tender and juicy and had more pork flavor than controls or those pumped with phosphate/salt only. Cegielska-Radziejewska and Pikul (2004) showed that SL inhibited the formation of malonaldehyde in sliced poultry sausage during refrigerated storage. Lamkey et al. (1991) reported that SL added to fresh pork sausage did not affect lean color (P>0.01) but resulted in more rapid surface discoloration (P < 0.01). Bradford et al. (1993) showed that 2% potassium lactate had no effect on sensory properties, TBAR and color L, a, b values of low-fat pork sausage or lean color during refrigerated aerobic storage. Choi and Chin (2003) showed that including 3.3% commercial SL in frankfurter formulation did not affect (P > 0.05) textural profile of the sausage. These results suggested that the effect of SL on the quality of products
depends on the SL level and product types.

High concentration of SDA has a negative effect on the flavor of ham products (Stekelenburg and Kant-Muermans, 2001). However, at lower levels (≤0.1%), SDA does not influence the quality of meat products (Stekelenburg, 2003).

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CHAPTER 3. THE ROLE OF DIETARY VITAMIN E IN EXPERIMENTAL LISTERIA MONOCYTOGENES INFECTIONS IN TURKEYS

A paper published in *Poultry Science* **

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**ABSTRACT:** The current study was designed to determine if dietary vitamin E influenced either the gut clearance or levels of peripheral blood CD4⁺ and CD8⁺ T lymphocytes in adult turkeys experimentally infected with *Listeria monocytogenes*. Turkeys were fed vitamin E (0, 100, or 200 IU) from day of hatch to time of necropsy. After 6 wk on the experimental diet, turkeys were orally inoculated with *L. monocytogenes* (×10⁹ cfu). To monitor infection status, cloacal swabs were taken on selected days post-inoculation (DPI). At necropsy, samples of viscera, including liver, spleen, cecum, duodenum, ileum, and colon were collected and cultured for *L. monocytogenes*. In experiments 1 and 2, recovery of *L. monocytogenes* from cloacal swabs, tissues, and intestines from turkeys fed vitamin E was generally lower than that from turkeys fed the control diet, although these differences were not statistically significant. When data from both trials were combined, *L. monocytogenes* was cultured less frequently from cloacal swabs of the vitamin E-treated group (200 IU) on 2 and 3 DPI, when compared to controls (0 IU, \( P < 0.01 \)). There were no changes in virulence characteristics of *L. monocytogenes* cells, as measured by in vitro killing of Ped-2E9 cells, recovered from cloacal swabs or tissues of experimentally infected turkeys fed the control or a vitamin E treatment.

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**Abbreviation Key:** DPI = days post-inoculation.
diet. Flow cytometric analysis indicated that CD4$^+$ and CD8$^+$ peripheral blood T lymphocytes were elevated at 6 and 8 DPI in infected turkeys given 200 IU vitamin E.

*(Keywords: CD4$^+$ and CD8$^+$, immune response, *Listeria*, turkey, vitamin E)*

**INTRODUCTION**

*Listeria monocytogenes* is a major human bacterial food-borne pathogen that annually accounts for ~ 2,500 cases (meningitis, encephalitis, sepsis, fetal death, prematurity) and 504 deaths (Mead et al., 1999). Sporadic human cases of listeriosis have been epidemiologically linked to the consumption of undercooked poultry products (Schwartz et al., 1988). Analysis of risk factors associated with sporadic human listeriosis in the U.S. indicated that cancer patients and immunocompromised patients, in whom 69% of listeriosis cases occur, were more likely than controls to have eaten undercooked poultry (odds ratio = 3.3; Schuchat et al., 1992). A 1998 multistate outbreak of human listeriosis, ascribed to serotype 4 (101 cases, resulting in 22 deaths), was linked to delicatessen meats, including turkey (MMWR, 1998). A 2002 outbreak involving 46 cases, seven deaths, and three stillbirths was linked to contaminated delicatessen turkey meats (MMWR, 2002). The recall of 26 million pounds of turkey meat in 2002 indicates the economic consequences of ready-to-eat meats contaminated with *L. monocytogenes* (U.S. Department of Health and Human Services, 2002).

Adult turkeys may be transiently colonized by consuming contaminated feed or water (Husu et al., 1990). Thus, *L. monocytogenes* may enter the packing plant at low levels in the intestine of recently infected birds, survive in bio-films, and ultimately contribute to both environmental and ready-to-eat product contamination (Genigeorgis et al., 1990; Ojeniyi et al., 1996). In the United States, *L. monocytogenes* was found on 5.9% of turkey carcass rinses and in 31% of ground turkey meat examined in the Nationwide Young Turkey Microbiological Baseline Data Collection Program (U.S. Department of Agriculture, 1998).

Vitamin E is required for normal development and function of the immune system in poultry (Boa-Am-ponsem et al., 2000; Leshchinsky and Klasing, 2000, 2001). In chickens, vitamin E supplement increased the number of lymphocytes in the bursa and the thymus gland and stimulated the proliferation and differentiation of T cells (Chang et al., 1994). In broilers,
vitamin E selectively increased the percentage of mature CD4+ T helper cells in the thymus and spleen but did not alter the percentage of thymic and splenic B cells and macrophages in total immune cell (Gore and Qureshi, 1997; Erf et al., 1998). Vitamin E enhanced immunity of birds to *Escherichia coli* infection, coccidiosis, infectious bursal disease, and Newcastle disease and altered cytokine expression in broilers (Tengerdy and Brown, 1977; Colnago et al., 1984; Erf et al., 1998; Leshchinsky and Klasing, 2000). However, vitamin E, although increasing serum levels of α-tocopherol, did not reduce the severity of *Eimeria maxima* infections in broilers (Allen and Fretterer, 2002).

Besides stimulating immune parameters, dietary vitamin E also enhances meat quality. Dietary vitamin E contributes to oxidative stability, extends shelf life and prevents oxidative off-odor of poultry meat, thus preserves the sensory quality of both frozen and refrigerated turkey breast meat (Ahn et al., 1997, 1998; Sheldon et al., 1997).

As part of the immune response during acute listeriosis, the host marshalls neutrophils, macrophages, natural killer cells, and T lymphocytes, especially CD4+ and CD8+ (Unanue, 2002). In vitro transfer experiments have shown that CD4+ and CD8+ cells are required to eliminate infection with wild-type strains of *L. monocytogenes* in mice (Kaufmann, 1993).

The current study was designed primarily to assess the effectiveness of dietary vitamin E, using doses previously shown to enhance meat quality, in accelerating the gut clearance of *L. monocytogenes* in experimentally infected adult turkeys. Secondarily, we monitored CD4+ and CD8+ T lymphocytes to evaluate the role of vitamin E as an immune potentiator.

**MATERIALS AND METHODS**

**Bacterial Inoculum**

*L. monocytogenes* (ATCC 700301) was obtained from American Type Culture Collection3. The stock cultures were maintained (−70°C) in 50% glycerol. For experimental inoculations, cultures of *L. monocytogenes* were grown on brain heart infusion agar with 20%

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3 American Type Culture Collection, Manassas, VA.
bovine blood and 0.5% yeast extract (30°C, 24 h, in 5% O2, 10% CO2, 85% N2) and harvested in PBS buffer (5 ml/plate). After centrifugation (5,000 x g, 10 min, 4°C), the pellet was washed twice with PBS and resuspended in 20 ml PBS. A 1-ml aliquot of the suspension was serially diluted in PBS. *L. monocytogenes* (cfu) were enumerated after incubation of brain heart infusion agar plates seeded with 0.1 ml of each serial 10-fold dilution (30°C for 24 h, in 5% O2, 10% CO2, 85% N2).

**Dietary Vitamin E**

DL-α-Tocopherol acetate (500 IU/g Rovimix E-50%)\(^4\), was used in the corn-soybean meal-based diet formulation, as described by Nam et al. (2003).

**Turkeys**

*Experiment 1.* One-day-old mixed sex Large White turkeys (n = 90) were obtained from a local hatchery and allotted to six rooms. Two rooms each (30 turkeys total) were randomly assigned to one of the three dietary treatments containing 0, 100, or 200 IU vitamin E/kg feed. After 5 wk, cloacal swabs were taken with sterile cotton-tipped applicators\(^5\) to ensure that birds were culture-negative for *Listeria*. Any turkeys positive for *Listeria* were eliminated prior to experimental inoculation. No attempt was made to select birds, which were innately resistant to *Listeria*. One week later, only *Listeria*-negative turkeys were orally challenged with 1 ml of *L. monocytogenes* (1 x 10\(^9\) cfu/ml). To monitor infection status, cloacal swabs were taken at 1, 4, 5, and 6 d post-inoculation (DPI). Turkeys (4 to 5 per group) were necropsied at 5, 8, 11, 14, and 25 DPI. Liver, spleen, cecum, duodenum, ileum, and colon from each bird were collected and processed for *Listeria* isolation as described below.

*Experiment 2.* One-day-old mixed sex Large White turkeys (n = 70) were obtained from a local hatchery and allotted to 4 rooms. Two rooms (35 turkeys total) were randomly assigned to diets containing either 0 or 200 IU vitamin E/kg feed. Prior to infection at 5 wk, cloacal swabs were taken and cultured for *Listeria*. At 6 wk, 30 *Listeria*-negative turkeys in each diet

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\(^4\)Roche Vitamins, Inc., Ames, IA.

\(^5\)Puritan Hardwood Products, Guilford, ME.
group were orally challenged with 1 ml of *L. monocytogenes* (1 × 10⁹ cfu/ml). The remaining five *Listeria*-negative turkeys in each diet group were moved to the clean pens and served as non-infected controls. To monitor infection status, cloacal swabs were taken at 1, 2, 3, 4, 6, 8, and 10 DPI. Turkeys (~5 per group) were necropsied at 2, 4, 6, 8, and 10 DPI. Liver, spleen, cecum, duodenum, ileum, and colon from each bird were collected and processed for *Listeria* isolation as described below.

**Bacterial Isolation**

To culture *Listeria*, cloacal swabs were placed in UVM I (10 ml) and incubated (2 to 3 d, 30°C, in 5% O₂, 10% CO₂, and 85% N₂). After enrichment, 100-μl UVM I enrichment was transferred into 10-ml *Listeria* secondary enrichment broth (UVM II) and incubated (30°C, in 5% O₂, 10% CO₂, and 85% N₂). After ~48 h, 100 μl of UVM II was plated to PALCAM *L. monocytogenes* selective agar (30°C, 5% O₂, 10% CO₂, and 85% N₂ for 48 h). At necropsy, the liver, spleen, cecum, duodenum, ileum, and colon from each bird were sampled, enriched in UVM I (10% wt/vol), and cultured as described above.

Two presumptive *Listeria* spp. colonies were recovered from each PALCAM agar plate and were verified as *L. monocytogenes* by a multiplex PCR assay as described (Wesley et al., 2002). A total of 245 isolates from cloacal swabs and viscera were stored (4°C) on tryptic soy agar slants supplemented with 0.6% yeast extract and assayed for virulence.

**Virulence Assay for *L. Monocytogenes* Isolates**

Single microcolonies from each of 245 *Listeria* isolates recovered from infected turkeys were tested in vitro for virulence, as described (Bhunia et al., 1994, 1995). The ratio of *L. monocytogenes* cells per each target hybridoma cell was approximately 1,000:1. Tissue culture plates were incubated (37°C and 7% CO₂) for 6 h prior to scoring Ped-2E9 cell death using a trypan blue exclusion assay. Percent hybridoma cell death at 6 h after microcolony challenge was calculated as follows: 

\[
\frac{[(\text{LNC} - \text{LLC})/\text{LNC}] \times 100}{\text{LNC}}
\]

where LNC = number of total live cells.

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⁶Oxoid Ltd., Basingstoke, Hampshire, UK.
⁷Becton Dickinson, Franklin Lakes, NJ.
Ped-2E9 cells observed in untreated or unchallenged control wells, and LLC = number of live Ped-2E9 cells in wells challenged with *Listeria* strain. Isolates that killed >70 to 90% Ped-2E9 cells within 6 h of challenge, which was comparable to the reference strains of *L. monocytogenes*, were scored as highly virulent. Isolates that killed <10% of Ped-2E9 cells, which was comparable to the 8 to 10.6% cell death observed for *L. innocua* (negative control), were scored as avirulent.

**Serum Vitamin E (α-Tocopherol) Analysis**

Blood samples (10 ml) were collected in serum separation vacutainer tubes. Serum vitamin E analyses were performed with a Hewlett Packard (HP) 6890 GC equipped with an on-column capillary injector and a FID detector. Serum vitamin E was calculated using an internal standard, 5a-cholestane, and expressed as micrograms per milliliter (Du and Ahn, 2002).

**Flow Cytometric Analysis of Lymphocyte Population**

Five milliliters of blood was collected from the wing vein into a vacutainer tube containing sodium heparin. The heparinized whole blood was transferred into a 15ml conical centrifuge tube containing 5 ml of fluorescence buffer (FB is PBS containing 1% heat-inactivated fetal blood serum and 0.05% NaN₃). The contents were mixed and centrifuged (200 g for 15 min, 4°C). The buffy coat was collected, washed three times with FB, and resuspended in 0.5 ml FB, as described (Stabel et al., 2000).

Direct dual color immunofluorescence staining was performed as described previously (Stabel et al., 2000). Briefly, 50 µl of buffy coat (~ 1 x 10⁶ viable cells) was incubated (20 min at room temperature) with 10 µl each of 1:20 diluted CT4-FITC (fluorescein isothiocyanate conjugate mouse anti-chicken CD4 monoclonal antibody; catalog number 8210-02) and CT8-PE (phycoerythrin mouse anti-chicken CD8a antibody; catalog number 8405-09). After

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8Hewlett Packard Co., Wilmington, DE.
9Southern Biotechnology Associates, Inc., Birmingham, AL.
fixation with 1.5% formalin, the fluorescence intensities were measured with a Becton-Dickson FACScan flow cytometer. Cells incubated with the fluorescently labeled isotype served as controls.

**Statistical Analysis**

A completely randomized design was used to examine the effects of *L. monocytogenes* recovery from cloacal swabs of infected turkeys on vitamin E supplemented (200 IU) and the control (0 IU) diets. The mean of serum vitamin E as well as immune cells were analyzed statistically by the general linear models procedure using SAS software. Student-Newman-Keuls’ multiple range test was used to compare differences among mean values (*P* < 0.05). Means and SEM are reported.

**RESULTS**

**Serum α-Tocopherol (Vitamin E)**

Dietary vitamin E resulted in an increase in serum α-tocopherol (vitamin E) for experiments 1 and 2 (Table 1). At the time of experimental inoculation with *L. monocytogenes* (wk 6), serum vitamin E levels in both the 100 IU and 200 IU vitamin E treatment groups were significantly elevated when compared to birds on the control diet (0 IU). For both trials 1 and 2, serum vitamin E titers were significantly elevated in birds receiving 200 IU when compared to controls (0 IU). Because serum vitamin E levels, at 8, 11, and 14 DPI, were consistently higher in turkeys fed 200 IU than that in turkeys receiving 100 IU vitamin E, only the 200 IU diet was evaluated in experiment 2.

**Recovery of *L. monocytogenes* in Cloacal Swabs**

In experiments 1 and 2, pre-inoculation cloacal swabs were negative for *L. monocytogenes*. In experiment 1 at 1 DPI, *L. monocytogenes* was detected in cloacal swabs of

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10 Becton Dickinson Co., Cockeysville, MD.

11 SAS Institute, Cary, NC.
turkeys (22/24, 91.7%) fed the control diet (0 IU vitamin E), as well as birds fed 100 IU (21/24, 87.5%) and 200 IU (17/25, 68%) vitamin E. At 4 DPI, *L. monocytogenes* was detected in swabs of 8.3% of control diet turkeys (2/24), whereas only one bird each was positive in the 100 IU (1/24, 4.2%) and 200 IU (1/25, 4%) treatment groups. On d 5 PI, three control birds (3/24, 12.5%) were positive, in contrast to none of the turkeys in the 100 IU and 200 IU groups. In experiment 2, at all days of post-infection sampling, *L. monocytogenes* was recovered more frequently from cloacal swabs of turkeys fed the control diet (0 IU) than in turkeys fed 200 IU vitamin E. When data from the two trials were combined (Figure 1), *L. monocytogenes* was cultured less frequently (*P* < 0.01) on d 2 and 3 in birds receiving 200 IU vitamin E when compared to control birds (0 IU).

**Recovery of *L. monocytogenes* in Tissues**

In experiment 1, *L. monocytogenes* was recovered more often in the ceca and ileum of control diet turkeys versus vitamin E-treated birds at 5 DPI. *L. monocytogenes* was not recovered from the intestine or tissue samples of turkeys on either control or vitamin E diets after 8 DPI (data not shown). For experiment 2, as summarized in Table 2, there were fewer tissue samples positive for *Listeria* in vitamin E-treated birds at 2, 4, or 6 DPI. At 8 DPI, *L. monocytogenes* was not recovered from any group (control and vitamin E).

**Virulence Assays for *L. monocytogenes* Isolates**

A total of 240 out of 245 isolates (98%) recovered from infected turkeys and submitted for virulence testing killed >70% to 90% of target hybridoma cells within 6 h of challenge. These were scored as highly virulent as were the inoculating strain (ATCC 700301) and the reference *L. monocytogenes* strains (positive controls). Of these 245 isolates, 156 were recovered from cloacal swabs and the remaining 89 were from intestinal tissues of turkeys. Four remaining isolates killed 3.8 to 12.8% of Ped-2E9 cells. This finding was comparable to the 8 to 10.6% cell death observed for *L. innocua* (negative control). These were scored as avirulent, and later were confirmed by PCR as isolates that were not *L. monocytogenes*. 
Flow Cytometric Analysis of Lymphocytes

As summarized in Figure 2, for experimentally infected birds, CD4⁺ populations of turkeys fed 200 IU vitamin E were increased ($P<0.05$) at 6, 8, 10, and 31 DPI when compared to infected turkeys fed control diets (0 IU vitamin E) (Figure 2a). At 6 and 8 DPI, the CD8⁺ T lymphocytes were higher ($P<0.05$) in infected turkeys given 200 IU vitamin E than in infected turkeys on control diets (0 IU vitamin E) (Figure 2b). CD4⁺CD8⁺ double positive lymphocytes of experimentally infected turkeys on the 200 IU vitamin E diet were also markedly elevated ($P<0.05$) at 6 and 8 DPI when compared to infected birds on the control (0 IU vitamin E) diet (Figure 2c).

DISCUSSION

The impact of dietary vitamin E on both gut colonization as well as on CD4⁺ and CD8⁺ T lymphocyte populations was evaluated in turkeys experimentally infected with L. monocytogenes. The vitamin E doses used were those previously shown to improve meat quality (Ahn et al., 1997, 1998). Serum vitamin E increased proportionately with dietary vitamin E content. This reflects effective gut absorption of vitamin E by the time of experimental challenge at the sixth week of dietary treatment. When cloacal swab data for the two trials were combined, L. monocytogenes was cultured less frequently ($P<0.01$) on d 2 and 3 in birds receiving 200 IU vitamin E when compared to control birds (0 IU). Vitamin E supplement was previously reported to have increased the resistance of mice to influenza virus as well as of chickens to Newcastle disease virus, and increased antibody production and phagocytosis in chickens infected with E. coli (Tengerdy and Brown, 1977; Franchini et al., 1991; Han et al., 2000).

No virulence differences of L. monocytogenes recovered from in birds receiving vitamin E were detected using the Ped-2E9 assay. The inoculating strains (ATCC 700301) and nearly 99% of the isolates recovered from cloacal swabs as well as from tissues, including spleen, liver, and intestine, were pathogenic for Ped-2E9 hybridoma cells after 6h of incubation.

In order to determine the role of dietary vitamin E on immune parameters, the lymphocytes of infected turkeys were analyzed. In this current study, dietary vitamin E (200
IU) was associated with elevation of CD4+ (6, 8, and 31 DPI), CD8+, as well as CD4+CD8+ T lymphocytes (6 and 8 DPI) in Listeria-infected turkeys, when compared with infected turkeys on control diets. This observation was in concert with the requirement of CD4+ and CD8+ T cells to eliminate listeriosis (Unanue, 2002).

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TABLE 1. Serum vitamin E levels (µg/ml) in turkeys fed 0, 100, 200 IU of vitamin E (experiment 1 and 2)

<table>
<thead>
<tr>
<th>Days post infection</th>
<th>0 IU</th>
<th>100 IU</th>
<th>200 IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.11±0.29c</td>
<td>2.08±0.33b</td>
<td>2.88±0.29a</td>
</tr>
<tr>
<td>8</td>
<td>0.16±0.20c</td>
<td>1.51±0.20b</td>
<td>3.10±0.20a</td>
</tr>
<tr>
<td>11</td>
<td>0.09±0.22c</td>
<td>1.75±0.22b</td>
<td>3.88±0.22a</td>
</tr>
<tr>
<td>14</td>
<td>0.15±0.21c</td>
<td>1.89±0.21b</td>
<td>3.67±0.22a</td>
</tr>
<tr>
<td>Experiment II</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>0.10±0.09b</td>
<td>3.42±0.24a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.10±0.08b</td>
<td>3.31±0.39a</td>
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</tr>
<tr>
<td>4</td>
<td>0.12±0.04b</td>
<td>3.19±0.29a</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.09±0.06b</td>
<td>3.41±0.35a</td>
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</table>

Means within a row with no or same letter (a, b, c) are not different (P > 0.05);
Four to five birds were analyzed at each sampling point for each dietary regimen.
TABLE 2. Recovery of *Listeria monocytogenes* from tissues of turkeys fed 0 and 200 IU of vitamin E (experiment 2)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Liver</th>
<th>Spleen</th>
<th>Cecal</th>
<th>Duodenum</th>
<th>Ileum</th>
<th>Colon</th>
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<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 IU</td>
<td>20%</td>
<td>0%</td>
<td>60%</td>
<td>40%</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>200 IU</td>
<td>40%</td>
<td>0%</td>
<td>60%</td>
<td>20%</td>
<td>20%</td>
<td>0%</td>
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<tr>
<td>Day 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 IU</td>
<td>0%</td>
<td>0%</td>
<td>60%</td>
<td>20%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>200 IU</td>
<td>0%</td>
<td>0%</td>
<td>60%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Day 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 IU</td>
<td>20%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>40%</td>
</tr>
<tr>
<td>200 IU</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>20%</td>
</tr>
</tbody>
</table>

1 Percentage of infected birds sampled for each day for each group, n = 5.
FIGURE 1. Comparison of the recovery of *Listeria monocytogenes* from cloacal swabs of turkeys fed control (0 IU) or a vitamin E (200 IU) diet. Significantly fewer turkeys harbored *L. monocytogenes* on d 2 and 3 post-infection (DPI) when fed vitamin E (200 IU). An asterisk (*) indicates a statistically significant difference between 0 IU (curve a) and 200 IU vitamin E (curve b) treatments at 2 and 3 DPI.
FIGURE 2. The response of CD4⁺, CD8⁺, and CD4⁺CD8⁺ populations, measured as percentage of lymphocytes, of turkeys fed 0 or 200 IU of vitamin E and experimentally infected with *Listeria monocytogenes*. The response was monitored for CD4⁺ (a), CD8⁺ (b), and CD4⁺CD8⁺ (c) lymphocytes. An asterisk (*) indicates a statistically significant difference between 0 IU and 200 IU vitamin E treatments. CD4⁺ populations of turkeys fed 200 IU vitamin E were higher at 6, 8, 10, and 31 d post-infection (DPI) when compared to infected turkeys fed a control diet (0 IU vitamin E) (b). At 6 and 8 DPI, the CD8⁺ lymphocytes were significantly higher in infected turkeys given 200 IU vitamin E than in infected turkeys on control diets (0 IU vitamin E). CD4⁺CD8⁺ double positive lymphocytes of experimentally infected turkeys on 200 IU vitamin E diet were also markedly elevated ($P<0.05$) at 6 and 8 DPI when compared to infected birds on the control (0 IU vitamin E) diet.
CHAPTER 4. EFFECT OF IRRADIATION ON THE QUALITY OF TURKEY HAM DURING STORAGE

A paper published in *Meat Science*

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**Abstract**

Effect of electron-beam irradiation on the quality of ready-to-eat (RTE) turkey ham was studied. Turkey hams were purchased from local stores and sliced into 0.5 cm-thick pieces and vacuum packaged. The ham samples were randomly separated into three groups and irradiated at 0, 1, or 2 kGy, and stored at 4°C for up to 14 days. Volatiles, color, TBARS values and sensory characteristics were determined to compare the effect of irradiation and storage on the quality of RTE turkey ham. Irradiation had little effects on color and TBARS values of RTE turkey hams. Sensory analysis indicated that sulfury odor increased as irradiation dose increased, and the contents of sulfur compounds in irradiated RTE turkey hams were higher (P < 0.05) than those in nonirradiated samples. Irradiation increased (P < 0.05) the production of acetaldehyde, which could be related to a metal-like flavor in irradiated hams. However, overall quality changes in RTE turkey hams by irradiation up to 2 kGy were minor.

Key words: Ready-to-eat turkey ham; Color; Flavor; Volatiles; Irradiation; Lipid oxidation

1. **Introduction**

Microbiological safety is a potential problem associated with ready-to-eat (RTE) meat

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products. Listeria monocytogenes, Salmonella and other pathogens may contaminate meat products during final packaging. The Food Safety and Inspection Service conducted a 10-year microbial prevalence study for RTE meat and poultry products produced from approximately 1,800 federally inspected establishments, and found that prevalence of Salmonella ranged from 0.05% to 1.43%; L. monocytogenes in sliced ham and luncheon meat was up to 5.16%, and other products ranged from 0.52% to 3.56%. None of the RTE products tested for E. coli O157:H7 or Staphylococcal enterotoxins was positive (Levine et al., 2001). This report clearly shows the seriousness of food safety problem in ready-to-eat meat products, especially for sliced ham.

Irradiation is the most effective technology in eliminating these pathogens in meat products. However, irradiation induces quality changes in meats such as off-odor production and color change, and consumer responses to these quality changes are quite negative (Ahn et al., 2000; Du et al., 2002; Jo & Ahn, 2000; Patterson & Stevenson, 1995). Due to the importance of irradiation in future meat industry, many studies were conducted to assess the impact of irradiation on the quality of raw or cooked meats. Most of the irradiation studies so far, however, are mainly focused on raw meat and cooked meat without additives, and little information on the quality changes of RTE meat products by irradiation are available. The objective of this study was to determine the effect of irradiation on the volatiles, sensory characteristics, color, and lipid oxidation of RTE turkey ham.

2. Materials and Methods

2.1. Sample preparation

Commercial turkey hams produced from a processor were purchased from local retail stores. Hams were sliced into 0.5 cm-thick pieces and vacuum (-1 bar of vacuum with 10 second dwell time) packaged in low oxygen-permeable bags (nylon/polyethylene, 9.3 mL O₂/m²/24 h at 0°C; Koch, Kansas City, MO). The sliced hams were randomly separated into three groups and electron-beam irradiated at 0 (control), 1, or 2 kGy using a Linear Accelerator (Circe IIIIR; Thomson CSF Linac, Saint-Aubin, France). The energy and power level used were 10 MeV and 10 kW, respectively, and the average dose rate was 88.3 kGy/min. To confirm the target dose, 2 alanine dosimeters per cart were attached to the top
and bottom surface of a sample. The alanine dosimeter was read using a 104 Electron Paramagnetic Resonance Instrument (Bruker Instruments Inc., Billerica, MA). The maximum were 1.15 kGy and 2.38 kGy; the minimum absorbed doses were 1.05 kGy and 2.02 kGy. After irradiation, turkey hams were stored at 4°C. Color, volatiles, and TBARS were analyzed at 0, 7 and 14 day, and sensory characteristics were determined after 5 day of storage.

2.2. Color measurement

The surface color of turkey hams was measured in package using a Hunter LabScan Colorimeter (Hunter Laboratory, Inc., Reston, VA) that had been calibrated against black and white reference tiles covered with the same packaging materials as used for samples. The CIE L*- (lightness), a*- (redness), and b*- (yellowness) values were obtained using an illuminant A (light source). One color reading was taken from each side of sliced ham.

2.3. 2-Thiobarbituric acid reactive substances (TBARS) measurement

Five grams of minced ham were weighed into a 50-ml test tube and homogenized with 50 µl butylated hydroxyanisole (7.2%) and 15 ml of deionized distilled water (DDW) using a Polytron homogenizer (Type PT 10/35, Brinkman Instruments Inc., Westbury NY, USA) for 15 s at high speed. One milliliter of the meat homogenate was transferred to a disposable test tube (13 x 100 mm), sulfanilamide (1% W/V, 20 µl) was added and mixed thoroughly. The samples were set at room temperature for 5 min and then thiobarbituric acid/trichloroacetic acid (15 mM TBA/15% TCA, 2 ml) was added. The mixture was vortex mixed and incubated in a boiling water bath for 15 min to develop color. Then samples were cooled in the ice-water for 10 min, mixed again, and centrifuged for 15 min at 2,500× g at 4°C. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 ml of DDW and 2 ml of TBA/TCA solution. The amounts of TBARS were expressed as milligrams of malonaldehyde per kilogram of meat (Zipser & Watts, 1962).
2.4. Volatiles analysis

A purge-and-trap dynamic headspace GC/MS system was used to identify and quantify the volatile compounds. One gram of minced turkey ham was placed in a 40-ml sample vial, and the vial was flushed with helium gas (99.999%) for 5 s at 40 psi. After capping with a Teflon-lined, open-mouth cap, the vial was placed in a refrigerated (4°C) sample tray. The maximum sample holding time in the sample tray before determination of volatiles was less than 5 h to minimize oxidative changes (Ahn, Jo, & Olson, 1999). Samples were heated to 40°C and purged with helium gas (40 ml/min) for 11 min. Volatiles were trapped with a Tenax/charcoal/silica trap column at 20°C, desorbed for 2 min at 220°C, concentrated using a cryofocusing unit at -90°C, then desorbed into a GC column for 60 s at 220°C. An HP-624 column (15 m, 250 μm i.d., 1.4 μm nominal), an HP-1 column (60 m, 250 μm i.d., 0.25 μm nominal), and an HP-Wax column (7.5 m, 250 μm i.d., 0.25 μm nominal) were combined using zero-volume connectors and used for volatile analysis. A ramped oven temperature was used: the initial oven temperature was set at 0°C for 2.5 min, then increased to 10°C at 5°C/min, to 45°C at 10°C/min, to 110°C at 20°C/min, to 210°C at 10°C/min, and held for 2.5 min). Liquid nitrogen was used to cool the oven below ambient temperature. Helium was the carrier gas at constant pressure of 22 psi. A mass selective detector (MSD) was used to identify and quantify volatile compounds in irradiated samples. The ionization potential of MS will be 70 eV, scan range was between 19.1 and 350 m/z. The identification of volatiles was achieved by comparing mass spectral data with those of the Wiley library and authentic standards whenever available. The peak area was reported as the amount of volatiles released.

2.5. Sensory evaluation

Twelve trained sensory panelists characterized the odor and flavor of irradiated hams. Panelists were selected based on interest, availability, and performance in screening tests conducted with samples similar to those being tested. Two training sessions were conducted. In the first session, panelists were asked to describe the sensory characteristics of irradiation odor and flavor, which were summarized into four attributes: sulfury, metal-like, oxidized,
and sweet. At the second session, panelists were trained to familiarize with those described attributes, the scale to be used, and with the range of intensities likely to be encountered during the study. A category scale (none, slightly, medium, strong, and extremely strong) was used to describe the sensory characteristics and was assigned a score ranging from 1 to 5 respectively. All samples presented in random order to panelists were labeled with random three-digit numbers. For sensory evaluation, panelists were first asked to smell samples and record the characteristics and intensity of smell perceived, and then taste samples for flavor characteristics.

2.6. Statistical analysis

Data were processed by the General Linear Model (GLM) of Statistical Analysis System (SAS, 2000). The differences in the mean values were compared by the SNK (Student-Newman-Keuls) multiple range test (P < 0.05), and mean values and standard error of the means (SEM) were reported.

3. Results and Discussion

3.1. Color values and TBARS values

Table 1 shows the surface color of turkey ham receiving different irradiation doses and storage time. Irradiation decreased color L* values at 0 and 14 days, but increased a* values at 0 and 14 days. However, no significant change in color was observed during storage, and the overall color changes were minor. This result was generally in agreement with previous reports, which indicated irradiation increased the redness of uncured raw and cooked meats (Luchsinger et al., 1996; Millar, Moss, & Stevenson, 2000; Nam & Ahn, 2002). The results of this study, however, showed that irradiation dose up to 2 kGy had minor effects on the color of turkey ham.

Irradiated turkey ham had significantly higher TBARS values than nonirradiated control at 0 day, but this difference disappeared after 7 and 14 days storage (Table 2). The increase in TBARS values after irradiation was small and should be related to the oxidation induced by free radicals generated by irradiation. Overall, irradiation only induced slight
change in TBARS values, which was expected due to the vacuum packaging conditions of turkey hams during irradiation and storage.

3.2. Sensory evaluation

According to the panel discussion, odor or flavor associated with irradiation off-odor/flavor were metal-like, oxidized, sulfur and sweet. The sensory attribute mostly strongly correlated with irradiation was sulfur odor/flavor (Table 3) and when ham was irradiated at 2 kGy was more intense (P<0.01) than nonirradiated control, but not different (P > 0.05) than that irradiated at 1kGy. A sweet, cooked corn like odor was constantly observed for irradiated raw meat (Ahn, Jo, & Olson, 2000). Hashim, Resurreccion, and McWatters (1995) evaluated the sensory characteristics of irradiated refrigerated and frozen chicken and found that irradiated raw chicken had higher fresh chickeny, bloody and sweet aroma intensities than nonirradiated samples. For cooked meat, sulfur-related odor/flavor and metal-like odor/flavor were observed previously (Ahn, Jo, & Olson, 2000; Du et al., 2002). The presence of sulfur and metal-like odor in irradiated samples was associated with the increased sulfur compounds and aldehydes in volatiles (Du et al., 2002).

3.3. Volatiles analysis

Table 4 showed the profile of volatiles of ham at 0 day of storage. The amount of total volatiles increased numerically as irradiation dose increased. Two sulfur compounds were detected in the volatiles of turkey ham, which were carbon disulfide and dimethyl disulfide. Carbon disulfide was not detected in nonirradiated hams, but detected in irradiated samples. The amount of dimethyl disulfide was significantly higher in irradiated ham than that in non-irradiation samples; furthermore, dimethyl disulfide was also greater for 2 kGy than for 1 kGy. This result was in agreement with sensory results where sulfur-related odor/flavor was significantly increased in irradiated samples. The content of acetaldehydes was also significantly higher in irradiated samples (Table 4). This could be related to the metal-like odor/flavor as perceived by sensory panelists. Du et al. (2002) suggested that acetaldehyde was related to metal-like odor in irradiated chicken rolls. The amounts of hexanal and pentanal in irradiated turkey hams were significantly greater than those in nonirradiated hams.
after 14 days of storage. Irradiation also increased 3-methyl butanal and 2-methyl butanal, which were associated with the radiolysis of leucine and isoleucine, respectively (Ahn, Jo, & Olson, 2000). The increases of alkanes and alkenes in hams after irradiation were also observed (Table 4 and Fig 1).

After 7 days of storage (Table 5), the contents of carbon disulfide, dimethyl disulfide, acetaldehyde and 1-heptene were still significantly higher in irradiated than nonirradiated turkey hams. After 14 days of storage (Table 6), the amount of sulfur compounds were still higher in irradiated than that in nonirradiated samples, but the difference in acetaldehyde disappeared.

The content of 1-propanol in volatiles of both nonirradiated and irradiated turkey hams were dramatically increased during storage time, and the 1-propanol value is significantly higher in non-irradiated ham. The reason for the increased 1-propanol was not clear, but could be related to bacterial growth during storage (Jorgensen, Huss, & Dalgaard, 2001).

During storage time, the content of aldehydes in nonirradiated samples increased significantly, but no significant changes were observed in irradiated samples (Fig 1a). The contents of alkanes and alkenes were decreased during storage, but the changes were not statistically significant due to relatively large variations (Fig 1c). Both alcohols and ketones in nonirradiated samples increased significantly during storage, but no change (P > 0.05) was observed in irradiation samples (Fig 1b, e). For each irradiation dose, the contents of sulfur compounds did not significantly change during storage (Fig 1d).

4. Conclusion

Our results showed that up to 2 kGy irradiation has limited effects on color and oxidation of vacuum-packaged commercial turkey ham; however, irradiation has significant influence on odor/flavor of vacuum-packaged turkey ham. Both sensory panelists and volatiles analysis showed that there were significant changes in sulfur-related odor/flavor in RTE turkey products by irradiation. Thus, future studies should be focused on the prevention of irradiation-induced flavor changes in RTE meat products.
Acknowledgement

This research was supported by the Midwest Poultry Consortium.

References


Table 1. Color values of irradiated turkey ham during storage

<table>
<thead>
<tr>
<th>Irradiation dose (kGy)</th>
<th>Storage day</th>
<th></th>
<th></th>
<th>SEM</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>( L^* )-value</td>
<td>61.1x</td>
<td>61.2</td>
<td>61.6x</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>60.3xy</td>
<td>61.2</td>
<td>61.2x</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>58.8y</td>
<td>59.6</td>
<td>59.2y</td>
<td>0.4</td>
</tr>
<tr>
<td>SEM</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>( a^* )-value</td>
<td>20.9x</td>
<td>20.7</td>
<td>20.7y</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>21.3x</td>
<td>21.0</td>
<td>20.7y</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>21.5y</td>
<td>21.1</td>
<td>21.6x</td>
<td>0.2</td>
</tr>
<tr>
<td>SEM</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>( b^* )-value</td>
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<td>15.0</td>
<td>14.8y</td>
<td>0.3</td>
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<td>15.2</td>
<td>15.4</td>
<td>15.3y</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>15.2</td>
<td>15.4</td>
<td>16.0x</td>
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<td>SEM</td>
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<td></td>
</tr>
</tbody>
</table>

Means within a column with no or same letter (x,y) are not different \( (P > 0.05) \); \( n = 8 \).
Table 2. TBARS of irradiated turkey ham during storage

<table>
<thead>
<tr>
<th>Irradiation dose</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>------</td>
<td>-------</td>
<td>--------</td>
<td>-----</td>
</tr>
<tr>
<td>0 kGy</td>
<td>0.60y</td>
<td>0.78</td>
<td>0.75</td>
<td>0.05</td>
</tr>
<tr>
<td>1 kGy</td>
<td>0.74x</td>
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<td>0.68</td>
<td>0.03</td>
</tr>
<tr>
<td>2 kGy</td>
<td>0.70x</td>
<td>0.72</td>
<td>0.78</td>
<td>0.05</td>
</tr>
<tr>
<td>SEM</td>
<td>0.02</td>
<td>0.06</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Means within a column with no or same letter (x, y) are not different ($P > 0.05$); n = 4.
Table 3. Sensory characteristics of irradiated turkey ham during storage

<table>
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<tr>
<th>Sensory characteristics</th>
<th>Irradiation dose (kGy)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Smell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metallic</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Oxidation</td>
<td>1.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Sulfury</td>
<td>1.7b</td>
<td>2.4ab</td>
</tr>
<tr>
<td>Sweet</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Flavour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metallic</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Oxidation</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Sulfury</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Sweet</td>
<td>2.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Means within a row with no or same letter (a, b) are not different (P > 0.05); n = 12.
Table 4. Volatile profiles of irradiated turkey hams at day 0 ($\times 10^4$ ion counts)

<table>
<thead>
<tr>
<th>Volatiles</th>
<th>0 kGy</th>
<th>1 kGy</th>
<th>2 kGy</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>2310c</td>
<td>6140b</td>
<td>10328a</td>
<td>1175</td>
</tr>
<tr>
<td>Pentane</td>
<td>0</td>
<td>460</td>
<td>749</td>
<td>236</td>
</tr>
<tr>
<td>Propanal</td>
<td>4342</td>
<td>6050</td>
<td>3464</td>
<td>1017</td>
</tr>
<tr>
<td>2-Propanone</td>
<td>34393</td>
<td>36327</td>
<td>34305</td>
<td>3271</td>
</tr>
<tr>
<td>Carbon disulfide</td>
<td>0b</td>
<td>10257ab</td>
<td>14499a</td>
<td>3486</td>
</tr>
<tr>
<td>Acetic acid methyl ester</td>
<td>2664</td>
<td>2073</td>
<td>2227</td>
<td>335</td>
</tr>
<tr>
<td>2-Methyl-propanal</td>
<td>871b</td>
<td>1727ab</td>
<td>2519a</td>
<td>388</td>
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<tr>
<td>Ethanol</td>
<td>1595</td>
<td>1535</td>
<td>2062</td>
<td>417</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>4421ab</td>
<td>3183b</td>
<td>6417a</td>
<td>764</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>15690</td>
<td>14957</td>
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<td>5253</td>
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<tr>
<td>Acetonitrile</td>
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<td>1694</td>
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<td>349</td>
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<tr>
<td>2,3-Butanedione</td>
<td>177b</td>
<td>230ab</td>
<td>286a</td>
<td>26</td>
</tr>
<tr>
<td>3-Methyl butanal</td>
<td>1330b</td>
<td>2680ab</td>
<td>3819a</td>
<td>453</td>
</tr>
<tr>
<td>2-Methyl butanal</td>
<td>1090</td>
<td>2284</td>
<td>2437</td>
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<tr>
<td>Benzene</td>
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<td>1217</td>
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<td>1-Heptene</td>
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<td>1138</td>
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Means within a row with no or same letter (a, b, c) are not different ($P > 0.05$); $n = 4$. 
Table 5. Volatile profiles of irradiated turkey hams after 7 days of storage ($\times 10^4$ ion counts)

<table>
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<td>98</td>
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Means within a row with no or same letter (a, b, c) are not different ($P > 0.05$); n = 4.
Table 6. Volatile profiles of irradiated turkey hams after 14 days of storage ($\times 10^4$ ion counts)

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<td>983</td>
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Means within a row with no or same letter (a, b, c) are not different ($P > 0.05$); n = 4.
Fig 1. Changes of major volatile groups from irradiated turkey hams during storage. Aldehydes include acetaldehyde, propanal, 2-methyl-propanal, 3-methyl-butanal, 2-methyl-butanal, pentanal, hexanal, 2-methyl-2-pentenal, and n-heptanal; Sulfur compounds include carbon disulfide and dimethyl-disulfide; Alkanes and alkenes include pentane, 1-heptene, 2,3,4-trimethyl-pentane, 2,3,3-trimethyl-pentane, octane, 2-octene, and 3-methyl-2-Heptene; Ketones include 2-propanone, 2-butanone, 2,3-butanedione, 2-pentanone, 4-methyl-2-pentanone, 3-methyl-2-pentanone, 3-hexanone, 2-hexanone and cyclopentanone; Alcohols include ethanol, 2-propanol, 1-propanol, and 2-butanol. a and b labeled on bars means there were significant difference ($P < 0.05$); $n = 4$. 
CHAPTER 5. INFLUENCE OF IRRADIATION AND STORAGE ON THE QUALITY OF READY-TO-EAT TURKEY BREAST ROLLS

A paper published in *Poultry Science* **


**ABSTRACT:** Influence of irradiation and storage on the quality of ready-to-eat (RTE) turkey breast rolls was investigated. Commercial oven roasted turkey breast rolls purchased from local stores were sliced and vacuum packaged. The sliced samples were randomly divided into three groups and irradiated at 0, 1.0, or 2.0 kGy using a linear accelerator. Color, 2-TBA reactive substances (TBARS), sensory characteristics, and volatiles were measured at 0, 7 and 14 d of storage. Irradiation increased color a*-value of turkey breast rolls. Irradiation and storage did not influence TBARS values. Sensory evaluation showed that irradiation significantly increased sulfury flavor. Because a dramatic increase in sulfur compounds was detected in irradiated samples, the sulfury flavor should be due to the sulfur compounds formed during irradiation. Irradiation also increased the amounts of acetylaldehyde, 2-methyl butanal, 3-methyl butanal, benzene, and toluene. It was concluded that irradiation significantly influenced the odor and flavor of RTE turkey breast rolls under vacuum packaging conditions. Therefore, strategies to prevent negative changes in the quality of irradiated RTE turkey breast roll are needed.

**Key words:** color, irradiation, ready-to-eat turkey breast roll, sensory, volatiles


**Dept. of Animal Science, *Dept. of Food Science and Human Nutrition Iowa State University**

**Primary researcher and author**

**Author for correspondence**
INTRODUCTION

Ready-to-eat (RTE) meat products are susceptible to environmental contamination with *L. monocytogenes* and other pathogens during handling of these products after cooking and before or during packaging. Recently, a nationwide recall of 27.4 million pounds of fresh and frozen RTE turkey and chicken products from a Wampler Foods Inc. that may have been contaminated with *Listeria monocytogenes* has caused a huge economic loss (FSIS-USDA, 2002). This indicates that there is an urgent need for an intervention to eliminate pathogens in the packaged products. Irradiation is an effective way of eliminating pathogens in RTE cooked meat products (Thayer, 1995; Savvaidis et al., 2002). Irradiation of packaged RTE meat products ensures the microbiological safety of these products, which in turn reduces costly product recalls and improves customer confidence in their safety. However, application of irradiation influences meat quality. The off-odor and color change induced by irradiation is positively related to irradiation dose (Ahn et al., 1999, 2000). Sensory panels detected irradiation off-flavor when the RTE turkey ham was irradiated at 2.0 kGy but not at 1.0 kGy (Zhu et al., 2003). Therefore, to prevent the quality change, a low dose of irradiation is preferred. At low dose, irradiation is still effective in killing pathogens, especially in combination with antimicrobial additives (Clavero et al., 1994; Sommers and Fan, 2003). Thus, a low irradiation dose was used in this study. Currently, limited information about the effects of irradiation on the quality of RTE turkey breast rolls is available. The objective of this study was to determine the effect of irradiation on the volatiles, sensory characteristics, color, and lipid oxidation in RTE turkey breast rolls.

MATERIALS AND METHODS

Sample Preparation

Commercial turkey breast rolls manufactured on the same day by a major poultry processor were purchased from local stores. The Breast rolls were sliced into 0.5 cm-thick pieces and vacuum packaged in low oxygen-permeable bags1 (nylon/polyethylene,

1Koch, Kansas City, MO
9.3 mL O2/m²/24 h at 0°C). The sliced breast rolls were randomly separated into three groups and electron-beam irradiated at 0, 1, or 2 kGy using a linear accelerator. The energy and power level used were 10 MeV and 10 kW, respectively, and the average dose rate was 88.3 kGy/min. To confirm the target dose, 2 alanine dosimeters were attached to the top and bottom surface of a sample. The alanine dosimeter was read using a 104 Electron Paramagnetic Resonance Instrument. The maximum were 1.15 kGy and 2.38 kGy; the minimum absorbed doses were 1.05 kGy and 2.02 kGy. After irradiation, RTE turkey breasts were stored at 4°C. Four packages of breast rolls per treatments were used for color, volatiles, and TBARS were analyzed at 0, 7 and 14 d respectively. Sensory characteristics were determined after 5 d of storage.

**Color Measurement**

The surface color of RTE turkey breasts was measured in package using a Hunter LabScan XE Colorimeter that had been calibrated against black and white reference tiles covered with the same packaging materials as used for samples. The CIE L* (lightness), a* (red/green axis, where -a points to green and +a extends to red), and b* (yellow/blue axis where -b points to blue and +b extends to yellow) values were obtained using an illuminant A (light source). One color reading was taken from each side of sliced breast.

**2-TBA Reactive Substances Measurement**

Five grams of minced breast were weighed into a 50-ml test tube and homogenized with 50 μl butylated hydroxyanisole (7.2%) and 15 ml of deionized distilled water (DDW) using a Polytron homogenizer (Type PT 10/35) for 15 s at high speed. One ml of the meat homogenate was transferred to a test tub (13 x 100 mm) and mixed with 2 ml thiobarbituric acid/ trichloroacetic acid (15 mM TBA/15% TCA). The mixture was incubated in a boiling

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2Circe IIIR; Thomson CSF Linac, Saint-Aubin, France
3Bruker Instruments Inc., Billerica, MA, USA
4Hunter Laboratory, Inc., Reston, VA
5Brinkman Instruments Inc., Westbury NY, USA
water bath for 15 min to develop color. Then sample was cooled in cold water for 10 min, and centrifuged for 15 min at 2,500×g at 4°C. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 ml of DDW and 2 ml of TBA/TCA solution. The amounts of 2-TBA reactive substances (TBARS) were expressed as milligrams of malonaldehyde per kilogram of meat (Ahn et al., 1997).

**Volatile Analysis**

Volatile of samples were analyzed using a Solatek 72 Multimatrix-Vial Autosampler /Sample Concentrator 3100 connected to a GC/MS (Model 6890/5973) according to the method of Ahn and Lee (2002). One gram of minced turkey breast was placed in a 40-mL sample vial, and the vial was flushed with helium gas (99.999%) for 5 s at 40 psi. After capping with a Teflon-lined, open-mouth cap, the vial was placed in a refrigerated (4 °C) sample tray. In order to minimize oxidative changes, the maximum sample holding time in the sample tray before determination of volatiles was less than 5 h (Ahn et al., 1999). Samples were heated to 40°C and purged with helium gas (40 mL/min) for 11 min. Volatiles were trapped with a Tenax/charcoal/silica trap column at 20°C, desorbed for 2 min at 220 °C, concentrated using a cryofocusing unit at −90 °C, then desorbed into a GC column for 30 s at 220 °C. A HP-624 column (15 m, 250 μm i.d., 1.4 μm nominal), a HP-1 column (60 m, 250 μm i.d., 0.25 μm nominal) and a HP-Wax column (7.5 m, 250 μm i.d., 0.25 μm nominal) were combined using zero-volume connectors and used for volatile analysis. A mass selective detector (MSD) was used to identify and quantify volatile compounds in irradiated samples. The authentic standard was used for identification whenever available. The peak area was reported as the amount of volatiles released.

**Sensory Evaluation**

Twelve trained sensory panelists characterized the smell and flavor of RTE turkey breasts. Panelists including faculties and students in the College of Agriculture were selected.

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6Tekmar-Dohrmann, Cincinnati, OH, USA
7Hewlett-Packard Co., Wilmington, DE, USA
based on interest, availability, and performance in screening tests conducted with samples similar to those being tested. Two training sessions were conducted. In the first session, panelists were asked to describe the sensory characteristics of irradiation odor and flavor, which were summarized into four attributes: sulfury, metallic, oxidized, and sweet. In the second session, panelists were trained to familiarize themselves with the described attributes, the scale to be used, and the range of intensities likely to be encountered during the study. A 5-point scale (1=none, 2=slightly, 3=medium, 4=strong, and 5=extremely strong) was used to describe the sensory characteristics. All samples were labeled with random 3-digit numbers and presented to panelists in random order. For sensory evaluation, panelists were first asked to smell samples and record the characteristics and intensity of smell perceived, and then tasted samples to determine flavor characteristics. Six samples were presented to panelists per session.

**Statistical Analysis**

A completely randomized design was used in this study. Individual package was considered as experimental unit. Four packages of samples were used for each analysis. Data were analyzed by the GLM procedure SAS (SAS Institute, 2000). The differences in the mean values were compared by the Tukey’s multiple comparison method (P < 0.05), and mean values and standard error of the means (SEM) were reported.

**RESULTS AND DISCUSSION**

**Color and TBARS values**

Irradiation at 1.0 kGy and 2.0 kGy did not significant affect color a* values at 0 and 7 d, and significantly increased a* values at 14 d (Table 1). During refrigerated storage, color a* values in 1.0- and 2.0 kGy- irradiated RTE turkey breast increased significantly at d 7 and 14, compared with those at d 0, this finding was in agreement with the redness changes of uncuried raw and cooked meats after irradiation (Luchsinger et al., 1996; Millar et al., 2000; Du et al., 2001; Nam and Ahn, 2002). In turkey ham, however, irradiation up to 2.0 kGy had only minor effects on color (Zhu et al., 2003), which could be associated with high intensity color of cured ham.
The TBARS values of RTE turkey breast did not change significantly at d 0 or after 14 d refrigerated storage (Table 2), because vacuum packaging prevented lipid oxidation during irradiation and refrigerated storage.

**Volatile and sensory evaluation**

Table 3 depicted the main volatile profiles of RTE turkey breast a few hours after irradiation and during storage. The production of acetaldehyde in all samples increased with storage time and irradiation dose. For the turkey breasts irradiated at 1.0 kGy, the amount of acetaldehyde at d 14 was significantly higher than that at d 0. For the turkey breasts irradiated at 2.0 kGy, the amount of acetaldehyde at d 7 and 14 was significantly higher than that of d 0. The amount of acetaldehyde in turkey breast irradiated at 2.0 kGy was significantly higher than that of 1.0 kGy and non-irradiated at d 7. At d 14, the amount of acetaldehyde in both 1.0- and 2.0 kGy-irradiated breast was significantly higher than that of non-irradiated samples. Acetaldehyde is suggested to give cooked meats a metallic flavor and was mainly derived from amino acid (Ahn, 2002). In this study, the sensory score for the metallic flavor increased numerically, but not significantly, as the irradiation dose increased, which should be associated with the increased acetaldehyde (Table 4). Irradiation also increased the production of 3-methyl butanal and 2-methyl butanal (Table 3), which are thought to be the radiolytic product of leucine and isoleucine, respectively (Ahn et al., 2000). There was no difference in hexanal content (Table 3), which is consistent with TBARS results (Table 2). Hexanal content in volatiles has been used as a marker for lipid oxidation and is related to TBARS values (Muguerza et al., 2003).

Irradiation with 1.0 and 2.0 kGy greatly increased the amount of dimethyl disulfide. The amount of dimethyl disulfide in turkey breast irradiated at 2.0 kGy was significantly higher than that treated at 1.0 kGy, which was significantly higher than that of non-irradiated breast (Table 3). Irradiation also induced other sulfur compounds but at lower amounts (data not shown). This is in agreement with the results from turkey ham (Zhu et al., 2003) and its sensory evaluation (Table 4). Sensory panelists noted that the sulfury odor and flavor of turkey breasts irradiated at 2.0 kGy were stronger than those of non-irradiated meat, but no difference was detected between samples irradiated at 1.0 kGy and non-irradiated controls.
Irradiation of RTE turkey breast, especially at 2.0 kGy, significantly increased the production of benzene. The production of toluene in turkey breast was increased but to a less extent. Because both benzene and toluene have negative effects on health (Pelclova et al., 2000; Bennett and Davis, 2002), their formation during irradiation warrants further study to investigate the formation of these compounds. Benzene and toluene maybe derived from packaging materials. However, benzene and toluene can also be generated from amino acids by irradiation (Ahn, 2002). Recent work in our laboratory showed that irradiation of meat products containing benzoate produced high amount of benzene (unpublished data). Thus, radiolysis of phenolic compounds might also contribute to the production of benzene in volatiles.

Table 4 shows the sensory scores of RTE turkey breasts with different irradiation doses. The intensities of metallic odor, oxidation odor, and sweet odor increased as irradiation dose increased, but the increase was not significant (Table 4). The sulfury odor and flavor of samples irradiated at 2.0 kGy were significantly higher (P < 0.01) than those of non-irradiated breasts. For turkey ham irradiated at 2.0 kGy, however, panelists could detect sulfury odor not flavor (Zhu et al., 2003), which could be due to the intensive cured flavor of turkey ham that masked the sulfur flavor. This result suggested that meat product such as turkey breast rolls, which do not have an intensive flavor, might need additional additives or other strategies to prevent the negative flavor induced by irradiation.

In conclusion, irradiation influences the quality of RTE turkey breast rolls. Results of sensory panelists and analysis of volatiles showed that irradiation, especially at 2.0 kGy, significantly influenced the odor and flavor of turkey breast rolls under vacuum packaging conditions. The formation of benzene and toluene during irradiation raises a concern about the chemical safety of irradiated RTE meats. Therefore, strategies to prevent negative changes in the quality of irradiated RTE turkey breast roll are needed.

**ACKNOWLEDGEMENT**

The research was supported by the Midwest Poultry Consortium. The NASA Food Technology Commercial Space Center purchased the Solartek 72 Multimatrix-Vial Autosampler used for volatile analysis in this study.
REFERENCES


TABLE 1. The surface color values (L*, a*, b*) of ready-to-eat (RTE) turkey breast under different irradiation dose and storage conditions (n=8)

<table>
<thead>
<tr>
<th>Irradiation dose</th>
<th>Storage day</th>
<th></th>
<th></th>
<th></th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>------</td>
</tr>
<tr>
<td><em><em>L</em>-value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>79.2</td>
<td>78.8</td>
<td>78.7(^a)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>1 kGy</td>
<td>78.3</td>
<td>78.0</td>
<td>77.4(^y)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>2 kGy</td>
<td>79.0</td>
<td>78.8</td>
<td>78.4(^xy)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em><em>a</em>-value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>9.3</td>
<td>10.1</td>
<td>10.0(^y)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>1 kGy</td>
<td>9.8(^b)</td>
<td>11.0(^a)</td>
<td>11.2(^ax)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>2 kGy</td>
<td>9.9(^b)</td>
<td>10.8(^a)</td>
<td>10.7(^ax)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em><em>b</em>-value</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>13.1(^b)</td>
<td>13.0(^b)</td>
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</tr>
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<td>14.5</td>
<td>13.1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>2 kGy</td>
<td>13.7</td>
<td>13.4</td>
<td>13.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.4</td>
<td>0.5</td>
<td>0.2</td>
<td></td>
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</tr>
</tbody>
</table>

\(^a\)Means within a row with no common superscript differ significantly \((P < 0.05)\).

\(^y\)Means within a column with no common superscript differ significantly \((P < 0.05)\).
TABLE 2. 2-Thiobarbituric acid reactive substances (TBARS) values of irradiated ready-to-eat (RTE) turkey breast during 14d of storage (n=4)

<table>
<thead>
<tr>
<th>Irradiation dose</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kGy</td>
<td>2.31</td>
<td>1.71</td>
<td>1.42</td>
<td>0.32</td>
</tr>
<tr>
<td>1 kGy</td>
<td>1.67</td>
<td>1.46</td>
<td>1.64</td>
<td>0.25</td>
</tr>
<tr>
<td>2 kGy</td>
<td>1.59</td>
<td>1.32</td>
<td>1.49</td>
<td>0.17</td>
</tr>
<tr>
<td>SEM</td>
<td>0.34</td>
<td>0.23</td>
<td>0.21</td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 3. The main volatiles (×10³ ion counts) from ready-to-eat (RTE) turkey breast 0, 7, and 14 d after irradiation (n=4)**

<table>
<thead>
<tr>
<th>Irradiation dose</th>
<th>Storage days</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>0 kGy</td>
<td>4198</td>
<td>6592</td>
</tr>
<tr>
<td>1 kGy</td>
<td>3934b</td>
<td>7922</td>
</tr>
<tr>
<td>2 kGy</td>
<td>6467b</td>
<td>15448</td>
</tr>
<tr>
<td>SEM</td>
<td>2033</td>
<td>2859</td>
</tr>
<tr>
<td><strong>Acetaldehyde</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>2696</td>
<td>2546</td>
</tr>
<tr>
<td>1 kGy</td>
<td>3080</td>
<td>3830</td>
</tr>
<tr>
<td>2 kGy</td>
<td>4322b</td>
<td>6089</td>
</tr>
<tr>
<td>SEM</td>
<td>632</td>
<td>506</td>
</tr>
<tr>
<td><strong>3-Methyl butanal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>1672</td>
<td>1527</td>
</tr>
<tr>
<td>1 kGy</td>
<td>1917</td>
<td>2314</td>
</tr>
<tr>
<td>2 kGy</td>
<td>2755b</td>
<td>3799</td>
</tr>
<tr>
<td>SEM</td>
<td>406</td>
<td>220</td>
</tr>
<tr>
<td><strong>2-Methyl butanal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>871z</td>
<td>1053</td>
</tr>
<tr>
<td>1 kGy</td>
<td>4930</td>
<td>5236</td>
</tr>
<tr>
<td>2 kGy</td>
<td>10486xa</td>
<td>9466</td>
</tr>
<tr>
<td>SEM</td>
<td>1171</td>
<td>533</td>
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<tr>
<td><strong>Dimethyl sulfide</strong></td>
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<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>8928</td>
<td>6814</td>
</tr>
<tr>
<td>1 kGy</td>
<td>7839</td>
<td>4137</td>
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<tr>
<td>2 kGy</td>
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<td>1171</td>
<td>533</td>
</tr>
<tr>
<td><strong>Hexanal</strong></td>
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<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>0y</td>
<td>0y</td>
</tr>
<tr>
<td>1 kGy</td>
<td>32y</td>
<td>54y</td>
</tr>
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<td>2 kGy</td>
<td>201x</td>
<td>244x</td>
</tr>
<tr>
<td>SEM</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td><strong>Benzene</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>179y</td>
<td>209y</td>
</tr>
<tr>
<td>1 kGy</td>
<td>433y</td>
<td>655y</td>
</tr>
<tr>
<td>2 kGy</td>
<td>971x</td>
<td>1177x</td>
</tr>
<tr>
<td>SEM</td>
<td>97</td>
<td>133</td>
</tr>
<tr>
<td><strong>Toluene</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>72213</td>
<td>55349</td>
</tr>
<tr>
<td>1 kGy</td>
<td>57385</td>
<td>62162</td>
</tr>
<tr>
<td>2 kGy</td>
<td>71986</td>
<td>93839</td>
</tr>
<tr>
<td>SEM</td>
<td>19174</td>
<td>8463</td>
</tr>
</tbody>
</table>

a,b: Means within a row with no common superscript differ significantly (P < 0.05).

x,y,z: Means within a column with no common superscript differ significantly (P < 0.05).
TABLE 4. Sensory characteristics of ready-to-eat (RTE) turkey breast among different irradiation treatments (n=12)

<table>
<thead>
<tr>
<th>Sensory Characteristics</th>
<th>Irradiation dose (kGy)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>------------------------</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td><strong>Odor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metallic</td>
<td>1.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Oxidative</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Sulfury</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweet</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Flavor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metallic</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Oxidative</td>
<td>2.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Sulfury</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweet</td>
<td>2.2</td>
<td>2.3</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with no common superscript differ significantly ($P < 0.05$).
CHAPTER 6. EFFECTS OF IRRADIATION ON SURVIVAL AND GROWTH OF
LISTERIA MONOCYTOGENES AND NATURAL MICROFLORA IN VACUUM-
PACKAGED TURKEY HAMS AND BREAST ROLLS

A paper submitted to Journal of Food Science

M.J. Zhu\textsuperscript{1,3}, A. Mendonça\textsuperscript{2,4}, H.A. Ismail\textsuperscript{1}, and D.U. Ahn\textsuperscript{1}

Abstract

The effect of electron-beam irradiation on the survival and growth of \textit{Listeria monocytogenes} and natural microflora in oven-roasted turkey breast rolls and turkey hams were evaluated. Slices of turkey breast rolls and hams were inoculated with a five-strain mixture of \textit{L. monocytogenes} to achieve $10^6$–$10^7$ CFU/cm\textsuperscript{2}, vacuum packaged, irradiated at 0 (control), 1.0, 1.5, 2.0 or 2.5 kGy, and stored at 4°C for up to 28 days. \textit{L. monocytogenes} (from inoculated samples) and aerobic plate count (APC) (from non-inoculated samples) were determined. Numbers of naturally-occurring bacteria on sliced turkey hams and breast rolls were 2.72 and 6.22 log\textsubscript{10} CFU/cm\textsuperscript{2}, respectively. The $D_{10}$-values of \textit{L. monocytogenes} in breast rolls and hams were 0.52 and 0.47 kGy, respectively. For breast rolls, the log\textsubscript{10} reductions of \textit{L. monocytogenes} following irradiation at 1.0 and 2.5 kGy were 1.5 and 4.7, respectively, while 2.0 and 5.5 for hams. The log\textsubscript{10} reductions of APC in breast rolls following 1.0 and 2.0-kGy irradiation were 2.9 and 5.2. For hams, the APC was reduced to $< 10$ CFU/cm\textsuperscript{2} after 1.0- and 2.0-kGy irradiation. In 2.0 kGy-irradiated hams, \textit{L. Monocytogenes} grew to 4.82 log\textsubscript{10} CFU/cm\textsuperscript{2} after 28 days of storage at 4°C, while APC increased to 2.98 log\textsubscript{10} CFU/cm\textsuperscript{2}, respectively. In breast rolls after 14 days of storage, APC increased to 2.98 log\textsubscript{10} CFU/cm\textsuperscript{2}, respectively. In breast rolls after 14 days of storage, APC in 1.0 kGy-irradiated samples increased to 7.53 log\textsubscript{10} CFU/cm\textsuperscript{2}; and APC increased to 2.63 and 4.68 log\textsubscript{10} CFU/cm\textsuperscript{2} for 2.0 kGy-irradiated breast rolls after 14 and 28 days of storage.

\textsuperscript{1}Dept. of Animal Science, \textsuperscript{2}Dept. of Food Science and Human Nutrition Iowa State University

\textsuperscript{3}Primary researcher and author

\textsuperscript{4}Author for correspondence
However, during the storage of breast rolls, *L. monocytogenes* grew slowly or even stopped to grow in both non-irradiated and irradiated breast rolls. The reason could be due to the competitive inhibition of natural flora in breast rolls. This study showed that irradiation (1.0 to 2.5 kGy) effectively reduced the number of *L. monocytogenes* and natural flora. However, *L. monocytogenes* and natural flora survived irradiation could multiply during 28 days of storage at 4 °C. Thus, additional hurdles are needed to ensure the microbial safety following low-dose irradiation.

**Keywords:** *Listeria monocytogenes*; natural flora; e-beam irradiation; ready-to-eat; turkey hams; turkey breast rolls

**Introduction**

*Listeria monocytogenes* is one of the pathogens that cause foodborne diseases most frequently, which accounts for about 2,500 cases and a loss of around $200 million annually. The mortality rate of clinical *Listeriosis* (~25%) is the highest of all foodborne illnesses (Mead and others, 1999). *L. monocytogenes* is commonly found in natural environment, the intestinal tract of infected animals, food processing environments and catering facilities (Gillespie and others, 2000; Beresford and others, 2001). In the United States, *L. monocytogenes* was found in 5.9% of turkey carcass rinses and in 31% of ground turkey meat (USDA, 1998). Due to its ubiquity in environment, it is challenging to prevent its transmission from raw animal products to meat processing environment onto ready-to-eat (RTE) meats (Tompkin and others, 1999). Further, *L. monocytogenes* that contaminate cured or non-cured RTE meat can increase to high numbers in these products during storage at refrigerator temperatures, which is due its resistance to low temperature and nitrite (Lou and Yousef, 1999). There have been three well-publicized outbreaks of *listeriosis* involving RTE meat products. A multistate outbreak in 1998 to 1999 was linked to frankfurters and deli meats and caused 101 cases and 21 deaths (CDC, 1999). In 2000, a multistate outbreak involving deli turkey meat results in 29 cases, 4 death and 3 miscarriages or still birth (CDC, 2000). More recently, an outbreak in the northeast United States was attributed to the consumption of sliceable turkey deli meat, and 46 confirmed cases, 7 deaths and 3 still births.
were associated with this outbreak (CDC, 2002). These outbreaks highlight the importance of preventing *L. monocytogenes* contamination in RTE meat products.

Irradiation has shown to be an effective way to eliminate pathogens, including *L. monocytogenes*. Most of those reports, however, were focused on assessing the effectiveness of gamma irradiation in reducing *L. monocytogenes* in poultry and red meats and other foods (Patterson and others, 1993; Gürel and Gürakan, 1997; Thayer and others, 1995; Thayer and others, 1998; Thayer and Boyd, 2000; Savvidis and others, 2002; Sommers and others, 2001; 2003). Electron beam irradiation is an emerging technology to control pathogen in foods, but few studies were conducted to determine the effectiveness of electron beam irradiation in eliminating *L. monocytogenes* (Fu and others, 1995; Shamsuzzaman and others, 1995; Tarte and others, 1996; Foong and others, 2004). The knowledge about recovery and subsequent growth of *L. monocytogenes* in RTE meat products after e-beam irradiation is also lacking (Foong and others, 2004). In this study, the effectiveness of electron beam irradiation on the survival and growth of *L. monocytogenes* and natural microflora in commercially available RTE turkey products were evaluated.

**Materials and Methods**

*Bacterial strains and growth conditions*

Five different *L. monocytogenes* strains (Scott A, H7969, H7596, H7762 and H7962) were used to inoculate sliced turkey hams and breast rolls. Prior to inoculation, each stock culture was individually grown in 10 ml Tryptic soy broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.6% yeast extract (Difco) (TSBYE) at 35 °C for 18 h. Then 1 ml of each strain was transferred individually to 100 ml of TSBYE and incubating at 35 °C for another 18 h. Each strain was harvested, washed twice, and re-suspended in sterile 0.1% (W/V) peptone (Difco) water. Inoculation cocktail was prepared by mixing equal volumes of the five strains suspension, which has approximately the same number of bacterial population.

*Preparation of meat samples*
Vacuum-packaged oven roasted turkey breast rolls and turkey hams were purchased
from local stores, sliced to 2-mm thick pieces in the meat laboratory, at Iowa State
University. Both turkey hams and breast rolls slices were randomly divided into two groups.
One group of the samples was used for microflora study and the other group for L.
monocytogenes inoculation study. Samples for microflora study were vacuum-packaged (one
slice per package) right after slicing, subdivided further into three groups, and then electron
beam irradiated at 0, 1.0 or 2.0 kGy using a Linear Accelerator (Circe IIIR; Thomson CSF
Linac, Saint-Aubin, France). The samples for L. monocytogenes inoculation study were
transferred to microbiology lab, surface inoculated with 0.1 ml L. monocytogenes cocktail
stock suspension to a level of $10^6$ CFU/cm$^2$, and then vacuum-packaged (1 slice per bag) in
nylon-polyethylene bags (3 mil standard barrier, $O_2 < 0.6cm^3/100in^2/24h$ at 38 °C; Koch
Industries, Kansas City, MO). The packaged samples were further separated randomly into
five groups and irradiated at 0 (control), 1.0, 1.5, 2.0, or 2.5 kGy using a Linear Accelerator
(Circe IIIR; Thomson CSF Linac, Saint-Aubin, France).

After irradiation, both inoculated and non-inoculated samples irradiated at 0, 1.0 and
2.0 kGy were stored at 4 °C for up to 28 days and sampled weekly. The number of natural
microflora in non-inoculated samples and L. monocytogenes in inoculated samples were
analyzed at each sampling day. The number of survived natural microflora following 0, 1.0,
2.0 kGy irradiation in non-inoculated samples and L. monocytogenes in 0, 1.0, 1.5, 2.0, or
2.5 kGy irradiated inoculated samples were analyzed at day 0.

**Microbiological analysis**

Each package was aseptically opened using an alcohol-sterilized scissors. Eight-five
milliliters of sterile 0.1% peptone was added to each meat sample (surface area ~85 cm$^2$)
followed by pummeling at medium speed for 1 min in a stomacher. Samples were serially
diluted with 0.1% peptone water and surface-plated (0.1 ml) in duplicate on modified oxford
(MOX) agar plates and tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) to
enumerate L. monocytogenes and background microflora, respectively. Listeria colonies on
MOX plates were counted after 48 h incubation at 35 °C. Natural microflora was counted
after 48 h incubation at 30 °C.
**Calculation of radiation $D_{10}$-values**

The number of survivors ($\log_{10} \text{CFU/cm}^2$) in inoculated sample at each irradiation level, based on colony counts from MOX plates, was plotted against irradiation dose to construct the survivor curves of *L. monocytogenes*. Least-squares analysis of the regression of the survivor values versus radiation dose was conducted. The $D_{10}$ value, radiation dose that results in 90% reduction of viable *L. monocytogenes*, was calculated as the reciprocal of the absolute value of the slope of the regression line.

**Results and Discussion**

In breast rolls, there was about 1.5 to 4.7 log reduction of *L. monocytogenes* after 1.0-to 2.5-kGy irradiation (Fig. 1). $\log_{10}$ reductions of *L. monocytogenes* in hams following 1.0-to 2.5-kGy irradiation ranged from 2.0 to 5.5. The $D_{10}$-value for turkey hams and breast rolls were about 0.47 and 0.52 kGy, respectively. The organism was a little more sensitive in turkey hams than turkey breast rolls, which could be associated with the differences in the formula of two products. In hams, sodium nitrite is included in formulation that could enhance the effectiveness of irradiation. Salt content in product also affects the effectiveness of irradiation in killing pathogenic organisms. Highly significant effects ($p < 0.01$) of water content, water activity and NaCl content on the survival of *Salmonella typhimurium* in irradiated mechanically deboned chicken meat and ground pork loin were observed (Thayer and others, 1995). Sommers and others (2003) reported that adding sodium diacetate (SDA) and potassium lactate (PL) increased the sensitivity of *L. monocytogenes* to gamma irradiation. Patterson (1989) showed that the $D_{10}$ values of *L. monocytogenes* in poultry meat by gamma irradiation were 0.42-0.55 kGy depending on strains and plating mediums, and the sensitivity of special strains of *L. monocytogenes* to irradiation varied with different meat substrates (Gürsel and Gürakan, 1997). Tarte and others (1996) reported that different strains of *L. monocytogenes* had different $D_{10}$ values, which ranged from 0.372 to 0.638 kGy in ground pork with e-beam irradiation.

Numbers of naturally-occurring bacteria on sliced turkey hams were $5.23 \times 10^2$ CFU/cm$^2$. During 28 days of refrigerated storage, aerobic plate count (APC) in non-irradiated turkey hams increased to $7.34 \log_{10}$ CFU/cm$^2$. Both 1.0- and 2.0-kGy irradiation
reduced natural microflora to < 10 CFU/cm² (Fig. 2). After 28 days of refrigerated storage, APC in 2.0 kGy-treated hams increased to 2.98 log₁₀ CFU/cm², equivalent to the starting APC level. For 1.0 kGy-irradiated samples, the equivalent starting APC level was achieved at 14 days. The numbers of natural flora on sliced turkey breast rolls were about 6.22 log₁₀ CFU/cm² (Fig. 3), which is quite high. After storage at 4 °C for 7 days, APC increased to 7.79 log₁₀ CFU/cm², and remained at this high level during 28 days of storage. Irradiation at 1.0 and 2.0 kGy reduced natural microflora to 3.33 and 0.98 log₁₀ CFU/cm², respectively. After irradiation, APC in 1.0 kGy-treated breast rolls increased rapidly, which increased to 7.53 log₁₀ CFU/cm² after 14 days of storage. The APC in 2.0 kGy-treated breast rolls increased to 4.68 after 28 days of storage.

Fig. 4 shows the viability of *L. monocytogenes* at 4 °C following irradiation at 0, 1.0 and 2.0 kGy in vacuum-packaged commercial oven roasted turkey hams that was artificially contaminated with *L. monocytogenes*. Irradiation at 1.0 and 2.0 kGy reduced *L. monocytogenes* by 1.89 and 3.91 log₁₀ CFU/cm², respectively. *L. monocytogenes* numbers in non-irradiated turkey hams increased about 1 log during the first 7 days of storage, then the organisms remained at peak population of 7-8 log₁₀ CFU/cm² during 28 days of storage at 4°C. No increase in numbers of survivors occurred in 1.0 kGy-irradiated turkey hams until after 7 days. In 2.0 kGy-irradiated turkey hams, the growth of *L. monocytogenes* was retarded for about 2 weeks. After 28 days of refrigerated storage, *L. monocytogenes* survivors in 1.0 kGy- and 2.0 kGy-treated turkey hams increased to 6.41 and 4.82 log₁₀ CFU/cm², respectively.

Fig. 5 showed the survival and growth of *L. monocytogenes* in irradiated and non-irradiated control breast rolls. One- and 2.0 kGy-irradiation reduced *L. monocytogenes* by 1.47 and 3.52 log₁₀ CFU/cm², respectively. No growth of *L. monocytogenes* was observed in both non-irradiation and 1.0 kGy-treated breast rolls during the whole 28 days of storage at 4°C. In 2.0 kGy-irradiated breast rolls, the number of *L. monocytogenes* survivors reduced by 1.35 log₁₀ CFU/cm² during the first 14 days of storage (Fig. 5), then *L. monocytogenes* grew slowly and the survivors increased to 2.71 log₁₀ CFU/cm² by the end of 28 days of storage (Fig. 5). This stationary behavior of *L. monocytogenes* in breast rolls during refrigerated storage should be due to high counts of natural microflora and the population.
composition of natural flora of the original samples (Fig. 3). The high counts of natural flora in RTE breast rolls might have a competitive advantage over *L. monocytogenes* for nutrient uptake, which inhibited their growth. It is also possible that the existing natural flora in breast rolls may alter RTE products pH and producing inhibitory metabolites, which inhibited the growth of *L. monocytogenes*. Lactic acid bacterial is usually the dominant microflora in meat products chill-stored under vacuum package (Holley and Mckellar, 1996), which have the antilisteria activity by producing bacteriocins and/or organic acids (Bredholt and others, 2001; Amezquita and Brashears, 2002; Mataragas and others, 2003). Further, irradiation can alter the dominant flora in meat products due to different irradiation sensitivity of different bacteria. Savvaidis and others (2002) showed that *Pseudomonas*, H$_2$S-producing bacteria typical of *S. putrefaciens*, and *Enterobacteriaceae* showed higher sensitivity to gamma irradiation than did the rest of the microbial species such as lactic acid bacteria. Thus, it is highly possible that lactic acid bacteria naturally occurring in this commercial RTE breast rolls inhibited *L. monocytogenes* multiplication during storage. Bredholt and others (2001) reported that the *Lactobacillus sakei* strain applied to cooked products at a concentration of 10$^5$-10$^6$ CFU/g immediately before slicing and vacuum-packaging inhibited growth of 10$^3$ CFU/g of a cocktail of three rifampicin-resistant mutant *L. monocytogenes* strains both at 8 °C and 4 °C. Bacteriostatic activity was observed in cooked hams, whereas bactericidal activity was observed in frankfurters (Amezquita and Brashears, 2002). Mataragas and others (2003) showed that the Listeria population in cooked cured pork shoulder inoculated with lactic acid bacteria, decreased by about 1.5 log$_{10}$ CFU/gram during 28 days of refrigerated storage, instead of increasing.

Fig. 6 showed the change of pH in vacuum-packaged turkey breast rolls and hams during storage. As shown in Fig. 6a, the pH for both irradiated and non-irradiated breast rolls dropped during storage. There were 0.76, 0.58 and 0.24 pH reduction for 0-, 1.0- and 2.0 kGy-irradiated breast rolls, respectively, indicating that pH reduction was correlated with the growth of microflora. During the 28-day storage, the pH of both 1.0- and 2.0 kGy-irradiated hams kept constant, while non-irradiated hams had 0.2 pH drop. This minor pH change of hams is in agreement with the low APC in hams.
Conclusion

Irradiation (1.0 to 2.5 kGy) greatly reduced *L. monocytogenes* and APC in turkey hams and breast rolls. However, from the D$_{10}$ value obtained in this study, at least 2.4 kGy and 2.6 kGy irradiation are needed to achieve a 5-log reduction of *L. monocytogenes* in turkey hams and breast rolls, respectively. Some cells survived irradiation and grew during storage after lag phase. To control *L. monocytogenes* contamination in RTE turkey hams and breast rolls during refrigerated storage, additional barriers, such as adding preservatives, are necessary in order to ensure the microbial safety of products following low-dose irradiation.

References


Fig. 1 Survival curves of *Listeria monocytogenes* in e-beam irradiated turkey hams and breast rolls
Fig. 2 Population of microflora in non-irradiated and irradiated vacuum-packaged turkey hams during 4°C storage
Fig. 3 Population of microflora in non-irradiated and irradiated vacuum-packaged oven roast turkey breast rolls during 4°C storage
Fig. 4 The growth of *L. monocytogenes* in irradiated and non-irradiated vacuum packaged turkey hams during 4°C storage.
Fig. 5 The growth of *L. monocytogenes* in irradiated and non-irradiated vacuum packaged turkey breast rolls during 4°C storage
Fig. 6 The change of pH in vacuum-packaged turkey breast rolls and hams during 4°C storage

a. Turkey breast rolls; b. Turkey hams
CHAPTER 7. EFFECTS OF ELECTRON BEAM IRRADIATION AND ANTIMICROBIALS ON THE VOLATILES, COLOR AND TEXTURE OF READY-TO-EAT TURKEY BREAST ROLL

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Abstract: Breast rolls with 6 antimicrobial additive treatments - no preservatives (control), 0.1% potassium benzoate (PB), 2% sodium lactate (SL), 0.1% potassium benzoate plus 2% sodium lactate (PB+SL), 2% sodium lactate plus 0.1% sodium diacetate (SL+SDA), and 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate (PB+SL+SDA) - were prepared. Samples were irradiated at 0, 1.0, or 2.0 kGy, and then the quality characteristics of turkey rolls were analyzed. Adding 2% SL increased the hardness, springiness, cohesiveness, chewiness, and resilience of breast rolls. Addition of PB or SDA, and irradiation had no significant effect on texture. Adding 2% SL affected color values. The color a* and b*values of turkey rolls with 2% SL added were significantly lower than those of the control, and this difference was maintained after irradiation and during storage. No difference in color and texture was observed between turkey rolls added with SL and those added with SL+PB+SDA. Breast rolls containing antimicrobials had more lipid oxidation than control. Irradiation and storage slightly enhanced lipid oxidation, although the overall lipid oxidation was very low. Irradiation promoted the formation of dimethyl disulfide and dimethyl trisulfide. Adding PB in breast rolls greatly increased the formation of benzene during irradiation, while other antimicrobial additives had no significant effects on volatiles.

Keywords: e-beam irradiation; sodium lactate; sodium diacetate; potassium benzoate; turkey breast roll; quality

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Introduction

The initial contamination with pathogenic microorganism and their proliferation during handling and storage directly compromise the safety of foods, especially in ready-to-eat meat (RTE) products which are consumed directly without further heating. Of those common food-borne pathogens, *Listeria monocytogenes* apparently poses the biggest threat due to its being ubiquitous in the environment and its ability to multiply during refrigerated storage (Beresford and others 2001).

Irradiation is an effective way to eliminate pathogens, including *L. monocytogenes*, *Salmonella*, *Yersinia enterocolitica*, and others (Patterson and others 1993; Thayer 1995; Tarte and others 1996; Thayer and Boyd 2001; Sommers and others 2002), but irradiation also causes quality changes in meat (Du and others 2002). Since the quality changes in irradiated meat are dose-dependent, only low-dosage irradiation is practiced by the meat industry in order to minimize those changes. The problem is that some pathogens can survive low-dose irradiation (Gürsel and Gürakan 1997; Clardy and others 2002). Those injured cells might repair themselves and then proliferate during storage at 4 °C (Gürsel and Gürakan 1997). Our result from RTE turkey ham and breast rolls showed that a significant number of inoculated *L. monocytogenes* survived low-dose irradiation and proliferated during refrigerated storage. These results suggest that additional hurdles are necessary in order to control bacteria growth during storage.

Sodium lactate, sodium diacetate, and potassium benzoate are extensively used to extend the shelf-life and ensure the safety of food products (Samelis and others 2001; Meyer and others 2003; Choi and Chin 2003). Byrne and others (2002) reported that the presence of 4% sodium lactate in beef burger patty formulations can reduce the overall risks posed to consumers by the presence of *E. coli O157:H7*, by reducing survival of the pathogen during frozen storage and increasing the susceptibility of the pathogen to heat during cooking. Sommers and Fan (2003) showed that adding sodium diacetate to sausages increased the sensitivity of *L. monocytogenes* to irradiation. The D10-values were 0.58, 0.59, 0.57, and 0.53 kGy for *L. monocytogenes* populations suspended in emulsions containing 0, 0.125, 0.25, and 0.5% SDA, respectively. Samelis and others (2001) found that the growth of *L. monocytogenes* inoculated on bologna slices was inhibited by dipping the meat in a solution
containing 5% potassium benzoate. In ready-to-eat meat products, addition of sodium lactate and acetate inhibits the growth of inoculated *L. monocytogenes* (Samelis and others 2002; Mbandi and Shelef 2002). Despite the effectiveness of antimicrobial additives in inhibiting the growth of microorganisms (Meyer and others 2003; Sommers and Fan 2003; Stekelenburg 2003), it has been shown that high concentration of antimicrobial additives, such as sodium diacetate, have a negative effect on the flavor of ham products (Stekelenburg and Kant-Muermans 2001). Currently, little is known about the effect of antimicrobial additives in combination with irradiation on the quality of RTE meat. Thus, the objective of this study was to determine the effect of antimicrobials and irradiation on the quality of turkey breast rolls.

**Materials and Methods**

**Preparation of RTE turkey meat products and irradiation**

Oven-roasted turkey breast rolls with different antimicrobial additives were freshly processed in the Meat Laboratory of Iowa State University. Six antimicrobial additive treatments were prepared, which included basic formula without any preservatives (control), with 0.1% (W/W) potassium benzoate (PB), 2% (W/W) sodium lactate (SL), 0.1% (W/W) potassium benzoate plus 2% (W/W) sodium lactate (PB+SL), 2% (W/W) sodium lactate plus 0.1% (W/W) sodium diacetate (SL+SDA), and 0.1% (W/W) potassium benzoate plus 2% (W/W) sodium lactate plus 0.1% (W/W) sodium diacetate (PB+SL+SDA). Each antimicrobial treatment and the basic meat ingredients (Table 1) were mixed with ground turkey breast for 3 min and stuffed into large fibrous casings (11.5 cm in diameter), which were clipped and stored overnight at 4 °C to facilitate cross-link formation by transglutaminase. Next day in the morning, the rolls were heat-processed in a smoke house to an internal temperature of 75 °C, immediately chilled with a cold water shower for 10 min, and stored at 4 °C for 4 h. The cooked chilled rolls were sliced (2.0-cm-thick slices for texture measurement and 1.0-cm-thick slices for other quality analyses) and vacuum-packaged. The vacuum-packaged breast slices from each additive treatment were randomly divided into 3 groups and irradiated at 0, 1.0, or 2.0 kGy using a Linear Accelerator (Circe
IIIR; Thomson CSF Linac, Saint-Aubin, France). The energy and power level used were 10 MeV and 10 kW, respectively, and the average dose rate was 88.3 kGy/min. To confirm the target dose, 2 alanine dosimeters were attached to the top and bottom surfaces of a sample. The alanine dosimeter was read using a 104 Electron Paramagnetic Resonance Instrument (Bruker Instruments Inc., Billerica, MA, USA). The max/min ratio for 1 kGy irradiation was 1.26 and that for 2 kGy irradiation was 1.17. After irradiation, RTE turkey breasts were stored at 4 °C up to 28 days. Color values, volatiles, and TBARS were analyzed at 0, 14, and 28 day. The texture characteristics were determined after 7 days of storage.

**Volatile analysis**

Volatile analysis of samples was conducted using a Solatek 72 Multimatrix-Vial Autosampler/ Sample Concentrator 3100 (Tekmar-Dohrmann, Cincinnati, OH, USA) connected to a GC/MS (Model 6890/5973; Hewlett-Packard Co., Wilmington, DE, USA) according to the method of Ahn and Lee (2002). One gram of minced turkey breast was placed in a 40-mL sample vial and the vial was flushed with helium gas (purity 99.999%) for 5 s at 40 psi. After capping with a Teflon-lined, open-mouth cap, the vial was placed in a refrigerated (4 °C) sample tray. The maximum sample holding time in the sample tray before determination of volatiles was less than 3 h to minimize oxidative changes (Ahn and others 1999). Samples were heated to 40 °C and purged with helium gas (40 mL/min) for 11 min. Volatiles were trapped with a Tenax/charcoal/silica trap column at 20 °C, desorbed for 2 min at 220 °C, concentrated using a cryofocusing unit at -90 °C, then desorbed into a GC column for 30 s at 220 °C. An HP-624 column (15 m, 250 μm i.d., 1.4 μm nominal), an HP-1 column (60 m, 250 μm i.d., 0.25 μm nominal), and an HP-Wax column (7.5 m, 250 μm i.d., 0.25 μm nominal) were combined using zero-volume connectors and used for volatile analysis. A mass selective detector (MSD) was used to identify and quantify volatile compounds in irradiated samples. The identification of volatiles was achieved by comparing mass spectral data with those of the Wiley library (Hewlett-Packard Co.) and authentic standards whenever available. The peak area was reported as the amount of volatiles released.

**2-Thiobarbituric acid-reactive substances (TBARS) measurement**
Five grams of minced breast were weighed into a 50-mL test tube and homogenized with 50 μL butylated hydroxyanisole (7.2%) and 15 mL of deionized distilled water (DDW) using a Polytron homogenizer (Type PT 10/35; Brinkman Instruments Inc., Westbury NY, USA) for 15 s at high speed. One mL of the meat homogenate was transferred to a test tube (13 x 100 mm) and mixed with 2 mL thiobarbituric acid/trichloroacetic acid (15 mM TBA/15% TCA). The mixture was incubated in a boiling water bath for 15 min to develop color. Then the sample was cooled in cold water for 10 min and centrifuged for 15 min at 2,500 x g at 4 °C. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL of DDW and 2 mL of TBA/TCA solution. The amounts of TBARS were expressed as mg malonaldehyde per kg meat (Ahn and others 1997).

Color measurement

The surface color of RTE turkey rolls (thickness =1.0 cm) was measured in package using a Hunter LabScan Colorimeter (Hunter Laboratory, Inc., Reston, VA) that had been calibrated against black and white reference tiles covered with the same packaging materials as used for the samples. The CIE L* (lightness), a* (redness), and b* (yellowness) values were obtained using an illuminant A (light source). One color reading was taken from each side of sliced breast, the average data were used for statistical analysis.

Texture profile analysis

The RTE turkey roll slices (2 cm in thickness) were immobilized between specially constructed stainless steel plates, a star-shaped, cheery-pitter probe (Texture Technology Corp., New York, NY, USA) to penetrate the slices perpendicularly. Each sample underwent 2 cycles of 50% compression using the above probe fitted to a TA-XT2i® Texture Analyzer (Texture Technology Corp.). Two separate texture profile analyses (TPA) were done per slice, and 4 slices were used for each treatment. Five textural parameters, hardness, cohesiveness, springiness, chewiness, and resilience, were obtained from the force-time curve and calculated as described by Bourne (1978).
Statistical analysis

A completely randomized design was used in this study. Four packages of samples were used for each analysis. Data were analyzed by the General Linear Model (GLM) of Statistical Analysis System (SAS 2000). The differences in the mean values were compared by the Tukey’s multiple comparison (P < 0.05), and mean values and standard error of the means (SEM) were reported.

Results and Discussion

Texture of RTE turkey breast rolls

Adding 2% SL to breast rolls significantly increased the hardness, springiness, cohesiveness, chewiness, and resilience of breast rolls (Table 2). PB (0.1%) and SDA (0.1%) addition had no significant effect on texture (Table 2). The increased values of texture parameters in 2% SL breast rolls probably due to the increased ionic strength, which increased the extraction of muscle proteins that helped aggregation of proteins when heated. Lavelle and Foegeding (1993) showed that salt was important for the turkey breast and thigh myofibrils to form good gelation with heating. Irradiation slightly increased the resilience of breast rolls of control, PB, SL, and SL+SDA (Table 2). The exact reason for this increase is not clear, but could be related to the cross-linking of amino acid residues by irradiation. In amino acid solutions, irradiation-induced cross-linking of amino acids and the solution turned turbid after irradiation (unpublished data).

Color of turkey breast rolls

Adding SL decreased the color a* and b* values of both irradiated and non-irradiated breast rolls and remained low during storage (Table 3, 4, 5). The change in color by SL could be related to the gelation of proteins. With 2% SL addition, the solubility of muscle proteins improved, which resulted in better gelation and changed the reflection of light on meat surface. Irradiation significantly increased the color a*-values (Table 3), but decreased the color b*-values of turkey rolls (Table 4). During storage, the color a*-values of irradiated breast rolls remained higher than those of nonirradiated samples (Table 3), while the color b*-

value remained lower (Table 4). There was no difference in the color L*- values among treatments (data not shown). The increase in the color a*-value after irradiation has been reported previously and was suggested to be associated with the CO formed during irradiation (Nam and others 2002).

**Lipid oxidation of breast rolls**

The TBARS values of nonirradiated breast rolls with antimicrobial additives added were slightly higher than those of the control at 0 day of storage (Table 5). Irradiation and storage increased TBARS values (Table 5), which could be due to the presence of residual oxygen or oxygen-permeating packaging material during storage. Electron beam irradiation is reported to accelerate lipid oxidation of meat under aerobic conditions (Du and others 2001) and the presence of salts might facilitate lipid oxidation in meat; however, the overall TBARS values were very low, since the breast rolls were made of high-quality raw turkey breast and were vacuum-packaged shortly after cooking, and the development of lipid oxidation was minor.

**Volatile s of breast rolls**

More than 40 different volatiles were detected (data not shown). Antimicrobial treatments had little effect on most of the volatiles from turkey breast rolls. Addition of PB, however, greatly increased the content of benzene in the volatiles of irradiated breast rolls (Figure 1). Benzene has deleterious effects on human health and is a potential carcinogen (Mehlman 2002; Bogadi-Sare and others 2003). Thus, PB may not be an ideal antimicrobial additive for irradiated meat products. Our previous study showed that benzene and toluene also could be formed from amino acids upon irradiation, possibly generated from aromatic amino acids (Ahn 2002).

Irradiation increased the amount of sulfur compounds, which has been shown in several previous studies (Du and Ahn 2002; Nam and others 2002; Nam and Ahn, 2003a). The content of dimethyl disulfide and dimethyl trisulfide increased greatly in irradiated samples (Figure 1). The contents of both sulfides were lower in samples containing SDA (SL+SDA and PB+SL+SDA) at 0 day of storage. After 28 days of storage, however, the sulfides in
PB+SL became lower than those of other treatments, which suggest that PB+SL might promote the degradation of dimethyl disulfide and dimethyl trisulfide. During the first 14 days of storage, the content of dimethyl trisulfide decreased significantly, while the content of dimethyl disulfide increased, which might be partially due to the degradation of dimethyl trisulfide to dimethyl disulfide. Nam and Ahn (2003b) also found that the amount of dimethyl disulfide increased during storage.

As reported previously (Du and Ahn 2002; Du and others 2002; Nam and Ahn 2003a, b), irradiation increased the amount of acetaldehyde and other aldehyde volatiles, alkanes, and alkenes (data not shown).

**Conclusion**

This study showed that including 2% SL affected the texture and color values of turkey breast rolls. The addition of PB in breast rolls produced a high amount of benzene after irradiation, suggesting benzoate salt is not a good antimicrobial to be used in products for irradiation. It also implies that certain spices or foods containing high amounts of phenolic compounds may not be suitable for irradiation. The combination of SL and SDA has a strong potential as an antimicrobial treatment for RTE meats, but low-dose irradiation (<2.0 kGy) is preferred due to side effects of irradiation.

**References**


Table 1. Formulation of oven-roasted turkey breast and pH in processed products

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Control</th>
<th>PB</th>
<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
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<td>Meat</td>
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<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Salt</td>
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<td>1.5</td>
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<td>Phosphate (Brifisol)</td>
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<td>Transglutaminase</td>
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<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Sodium caseinate</td>
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<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>Dextrose</td>
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<td>0.5</td>
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<td>0.5</td>
<td>0.5</td>
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<td>Water</td>
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<td>Sodium diacetate</td>
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<td>0.1</td>
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| pH              | Breast roll | 6.38| 6.38| 6.37| 6.38| 6.34| 6.35 |

Control = basic formula, PB = including 0.1% potassium benzoate, SL = including 2% sodium lactate, PB+SL = including 0.1% potassium benzoate and 2% (W/W) sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Table 2. Texture data for irradiated breast rolls with antimicrobial additives

<table>
<thead>
<tr>
<th>Irradiation dose (kGy)</th>
<th>Control</th>
<th>PB</th>
<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
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<tbody>
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<td>4.085ax</td>
<td>4.160a</td>
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<td>3.466bcy</td>
<td>4.087a</td>
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<td>3.137bc</td>
<td>3.918a</td>
<td>3.850a</td>
<td>3.381abc</td>
<td>3.566ab</td>
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<tr>
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<td>0.076</td>
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**Hardness**

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<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0.766c</td>
<td>0.759c</td>
<td>0.832a</td>
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<td>0.764c</td>
<td>0.751c</td>
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<td>0.802ab</td>
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<td>0.753c</td>
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<td>0.812a</td>
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<tr>
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<td>0.005</td>
<td>0.005</td>
<td>0.0052</td>
<td>0.006</td>
<td>0.004</td>
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**Springiness**

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<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
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<tr>
<td>0</td>
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<td>0.659a</td>
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<td>0.636a</td>
<td>0.647a</td>
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<td>0.005</td>
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**Cohesiveness**

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<th>Control</th>
<th>PB</th>
<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
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<td>2.082a</td>
<td>1.776b</td>
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<td>2.155ax</td>
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<td>1.501c</td>
<td>1.394c</td>
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<td>1.781by</td>
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**Chewiness**

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<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
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</thead>
<tbody>
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<td>0.177xy</td>
<td>0.279ay</td>
<td>0.225b</td>
<td>0.272ax</td>
<td>0.260a</td>
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<td>0.224c</td>
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<td>0.008</td>
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</table>

**Resilience**

Samples were stored at 4 °C for 7 days before texture measurement. n=4.

abc Means within a row with different superscript differ significantly (P < 0.05).

XYZ Means within a column with different superscript differ significantly (P < 0.05).

Control= basic formula, PB= including 0.1% potassium benzoate, SL= including 2% sodium lactate, PB+SL= including 0.1% potassium benzoate and 2% (W/W) sodium lactate, SL+SDA= including 2% sodium lactate and 0.1% sodium diacetate, PSS= including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Table 3. Color a* values for irradiated breast rolls with antimicrobial additives

<table>
<thead>
<tr>
<th>Irradiation dose (kGy)</th>
<th>Control</th>
<th>PB</th>
<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
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<td>5.07&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>4.91&lt;sup&gt;by&lt;/sup&gt;</td>
<td>5.14&lt;sup&gt;by&lt;/sup&gt;</td>
<td>0.14</td>
</tr>
<tr>
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<td>6.99&lt;sup&gt;abx&lt;/sup&gt;</td>
<td>6.34&lt;sup&gt;by&lt;/sup&gt;</td>
<td>6.66&lt;sup&gt;aby&lt;/sup&gt;</td>
<td>6.61&lt;sup&gt;abx&lt;/sup&gt;</td>
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<td>7.03&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>7.39&lt;sup&gt;abx&lt;/sup&gt;</td>
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<td>5.52&lt;sup&gt;x&lt;/sup&gt;</td>
<td>5.67&lt;sup&gt;y&lt;/sup&gt;</td>
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<td>0.13</td>
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<sup>abc</sup> Means within a row with different superscript differ significantly (<i>P &lt; 0.05</i>). n=4

<sup>xyz</sup> Means within a column with different superscript differ significantly (<i>P &lt; 0.05</i>).

Control = basic formula, PB = including 0.1% potassium benzoate, SL = including 2% sodium lactate, PB+SL = including 0.1% potassium benzoate and 2% (W/W) sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Table 4. Color b* values for irradiated breast rolls with antimicrobial additives

<table>
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<th>Irradiation dose (kGy)</th>
<th>Control</th>
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<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
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</tr>
<tr>
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<td>0.15</td>
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<sup>abc</sup>Means within a row with different superscript differ significantly (P < 0.05). n = 4

<sup>xy</sup>Means within a column with different superscript differ significantly (P < 0.05).

Control= basic formula, PB= including 0.1% potassium benzoate, SL= including 2% sodium lactate, PB+SL= including 0.1% potassium benzoate and 2% (W/W) sodium lactate, SL+SDA= including 2% sodium lactate and 0.1% sodium diacetate, PSS= including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Table 5. TEARS\textsuperscript{1} values for irradiated breast rolls with antimicrobial additives

<table>
<thead>
<tr>
<th>Irradiation dose (kGy)</th>
<th>Control</th>
<th>PB</th>
<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>0.029\textsuperscript{dz}</td>
<td>0.049\textsuperscript{aby}</td>
<td>0.048\textsuperscript{by}</td>
<td>0.038\textsuperscript{bcy}</td>
<td>0.061\textsuperscript{az}</td>
<td>0.060\textsuperscript{avy}</td>
<td>0.003</td>
</tr>
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<td>1.0</td>
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<td>0.087\textsuperscript{bcy}</td>
<td>0.078\textsuperscript{x}</td>
<td>0.158\textsuperscript{ax}</td>
<td>0.135\textsuperscript{abx}</td>
<td>0.100\textsuperscript{bcxy}</td>
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</tr>
<tr>
<td>2.0</td>
<td>0.104\textsuperscript{bx}</td>
<td>0.162\textsuperscript{ax}</td>
<td>0.091\textsuperscript{x}</td>
<td>0.139\textsuperscript{abx}</td>
<td>0.110\textsuperscript{by}</td>
<td>0.129\textsuperscript{abx}</td>
<td>0.011</td>
</tr>
<tr>
<td>SEM</td>
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<td>0.011</td>
<td>0.004</td>
<td>0.015</td>
<td>0.004</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 days</td>
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<tr>
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<td>0.180\textsuperscript{ax}</td>
<td>0.123\textsuperscript{d}</td>
<td>0.103\textsuperscript{ex}</td>
<td>0.134\textsuperscript{cx}</td>
<td>0.138\textsuperscript{ex}</td>
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</tr>
<tr>
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<td>0.141\textsuperscript{avy}</td>
<td>0.113\textsuperscript{bc}</td>
<td>0.105\textsuperscript{cx}</td>
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<td>0.080\textsuperscript{dy}</td>
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<td>0.070\textsuperscript{dy}</td>
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<tr>
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<td>0.004</td>
<td>0.005</td>
<td>0.003</td>
<td>0.003</td>
<td>0.002</td>
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<td>28 days</td>
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<td>0.183\textsuperscript{d}</td>
<td>0.185\textsuperscript{cdx}</td>
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<tr>
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<td>0.144\textsuperscript{ez}</td>
<td>0.213\textsuperscript{ax}</td>
<td>0.190\textsuperscript{bc}</td>
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</tr>
<tr>
<td>SEM</td>
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<td>0.002</td>
<td>0.006</td>
<td>0.002</td>
<td>0.002</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{abc} Means within a row with different superscript differ significantly \((P < 0.05). n = 4\)

\textsuperscript{xyz} Means within a column with different superscript differ significantly \((P < 0.05)\).

\textsuperscript{1}The TBARS was expressed as milligrams of malonaldehyde per kilogram of meat.

Control = basic formula, PB = including 0.1% potassium benzoate, SL = including 2% sodium lactate, PB+SL = including 0.1% potassium benzoate and 2% (W/W) sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Figure 1. Benzene, dimethyl disulfide, and dimethyl trisulfide contents in the volatiles of irradiated breast rolls with or without antimicrobial additives

Means within group with different superscript differ significantly ($P < 0.05$). $n = 4$

BF = basic formula, PS = including 0.1% potassium benzoate and 2% (W/W) sodium lactate, SS = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
CHAPTER 8. FATE OF LISTERIA MONOCYTOGENES IN READY-TO-EAT TURKEY BREAST ROLLS FORMULATED WITH ANTIMICROBIALS FOLLOWING E-BEAM IRRADIATION

A paper submitted to Journal of Food Protection

M.J. Zhu\textsuperscript{1,3}, D.U. Ahn\textsuperscript{1}, H.A. Ismail\textsuperscript{1}, M. Du\textsuperscript{1}, and A. Mendonca\textsuperscript{2,4}

Abstract: The objective of this study was to determine the effect of antimicrobials on the survival and proliferation of \textit{L. monocytogenes} in turkey breast rolls following electron-beam irradiation. Six antimicrobial additive treatments that include no preservatives (control), 0.1% potassium benzoate (PB), 2% sodium lactate (SL), 0.1% potassium benzoate plus 2% sodium lactate (PB+SL), 2% sodium lactate plus 0.1% sodium diacetate (SL+SDA), and 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate (PB+SL+SDA) were used. Sliced turkey breast rolls were artificially inoculated with \(10^6\) CFU/cm\textsuperscript{2} five-strain-\textit{L. monocytogenes} cocktails, then vacuum-packaged and irradiated at 0, 1.0, 1.5, 2.0 or 2.5 kGy. \(D_{10}\) values for breast rolls with various additive treatments ranged from 0.56 to 0.58 kGy. Adding PB (0.1%) or SL (2%) in turkey rolls failed to prevent \textit{L. monocytogenes} from growing during refrigerated storage. In turkey rolls added with two (PB+SL or SL+SDA) or three (PB+SL+SDA) antimicrobial combinations had 2 or 3 weeks of lag phases before \textit{L. monocytogenes} growth, respectively. Irradiating turkey rolls, which were added with PB+SL or SL+SDA, at 1.0 kGy was effective in suppressing the growth of \textit{L. monocytogenes} for about six weeks when stored at 4 °C. No growth of \textit{L. monocytogenes} after irradiation occurred during 42 days of storage for 2.0 kGy irradiated breast rolls formulated with 0.1%PB+2%SL, 2%SL+0.1%SDA or 0.1%PB+2%SL+0.1%SDA, and 1.0 kGy irradiated

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\textsuperscript{3} Primary researcher and author
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turkey breast with 0.1% PB + 2% SL + 0.1% SDA. Sensory panelists found that low-dose irradiation (1.0 kGy) had no effect on the sensory characteristics of RTE turkey breast rolls. Including SL+SDA had slightly negative effect for nonirradiated turkey breast rolls, but the sensory characteristics of 1.0 kGy irradiated turkey roll containing SL+SDA was not significantly different from the others receiving 1.0kGy irradiation. For microbiology safety, PB+SL and SL+ SDA antimicrobial treatments combined with 1.0 kGy or 2.0 kGy irradiation are a promising technology.

**Keywords:** *Listeria monocytogenes*; e-beam irradiation; sodium lactate; sodium diacetate; potassium benzoate; turkey breast roll

**INTRODUCTION**

Due to its high mortality rate (~ 25%) and economic losses caused by expensive product recalls (Mead *et al.*, 1999; U.S. Department of Health and Human Services, 2002), *Listeria monocytogenes* is a big food safety issue for the processed meat industry. For RTE meat products, the most frequently applied hurdles such as thermal processing, vacuum packaging, refrigerated storage and nitrite seem insufficient when it comes to *L. monocytogenes* due to its ubiquitous nature (Beresford *et al.*, 2001), ability to grow at refrigerated temperature and anaerobic condition, and resistance to salt and nitrite (Lou and Yousef, 1999). Although *L. monocytogenes* can be killed during the thermal processing of RTE meats (Lemaire *et al.*, 1989; Zaika *et al.*, 1990), post-processing contamination of RTE meat with *L. monocytogenes* during slicing and packaging is difficult to avoid. To ensure microbiological safety of RTE meat, it is essential to have additional hurdles to control the growth of pathogen during refrigerated storage.

Formulating meat products with antimicrobial additives are one approach to suppress the growth of contaminated *L. monocytogenes* during storage, but they can not destroy the pathogenic organisms that existed in RTE-meat. Furthermore, including high concentration of antimicrobials such as SDA has a negative effect on the flavor of meat products (Stekelenburg and Kant-Muermans, 2001). Food irradiation is an effective post-packaging intervention technology to eliminate those contaminated *L. monocytogenes* in RTE meat
products (Patterson et al., 1993; Thayer, 1995; Tarte et al., 1996; Thayer and Boyd, 2000; Clardy et al., 2002; Foong et al., 2004). Due to its negative effects on meat quality, only low dosages of irradiation are recommended in RTE meats (Zhu et al., 2003; 2004a). However, pathogens survived at low-dose irradiation could repair themselves, proliferate and cause a health hazard during refrigerated storage (Gursel et al., 1997; Foong et al., 2004), which suggested additional hurdles besides low-dose irradiation were necessary.

Antimicrobials were used in combination with irradiation to suppress the growth of L. monocytogenes following irradiation. Gamma irradiation of L. monocytogenes suspended in SDA resulted in synergistic reductions of the microorganism, and supplementing SDA in beef bologna inhibited the proliferation of L. monocytogenes, which survived the irradiation process (Sommers and Fan, 2003). Gamma irradiation at 3.0 kGy prevented the proliferation of L. monocytogenes and background microflora in bologna containing 0.07% SDA and 1% potassium lactate, and in bologna containing 0.15% SDA and 2% potassium lactate over 8 weeks of storage at 9 °C (Sommers et al., 2003). We found that turkey hams formulated with 2% SL+0.1% SDA and 0.1% potassium benzoate (PB) +2% SL in combination with 1.0 kGy e-beam irradiation was effective in suppressing the growth of L. monocytogenes for about six weeks at 4 °C, and 2.0 kGy irradiation was listeriostatic (Zhu et al., 2004c). No studies were conducted to assess the effect of irradiation in combined with antimicrobials on the growth of L. monocytogenes in uncured turkey breast rolls, where L. monocytogenes may behave differently.

In the current study, potassium benzoate, sodium lactate and sodium diacetate alone or in combination were tested for their ability in inhibiting the growth of L. monocytogenes in RTE turkey breast rolls following 1.0 kGy or 2.0 kGy e-beam irradiation during 4°C storage. The effects of antimicrobial additives and irradiation on the sensory characteristics of turkey breast rolls were also assessed.

MATERIALS and METHODS

Bacterial strains and growth conditions

Five different L. monocytogenes strains (Scott A, H7969, H7596, H7762 and H7962) were used to inoculate sliced turkey breast rolls. Prior to inoculation, each stock culture was
individually grown in 10 ml Tryptic soy broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.6% yeast extract (Difco) (TSBYE) at 35 °C for 18 h. Then 1 ml of each strain was transferred individually to 100 ml of TSBYE and incubated at 35 °C for another 18 h. Each strain was harvested, washed twice, and re-suspended in sterile 0.1% (W/V) peptone (Difco) water. Inoculation cocktail was prepared by mixing equal volumes of the five strains suspension, which has approximately the same number of bacterial population.

Preparation of RTE turkey meat products

Oven roast turkey breast rolls with different antimicrobial additives were freshly processed in the Meat Lab at Iowa State University. Six antimicrobial additive treatments that include basic formula without any preservatives (control), with 0.1% potassium benzoate (PB), with 2% sodium lactate (SL), with 0.1% potassium benzoate and 2% sodium lactate (PB+SL), with 2% sodium lactate and 0.1% sodium diacetate (SL+SDA), or with 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate (PB+SL+SDA) were mixed with meat and other ingredients and then stuffed into large fibrous casings (ϕ=11.5cm). The rolls were heat processed to 74 °C internal temperature in an 84°C smoke house, chilled (4 °C), sliced to 2-mm-thick pieces and used for microbiological study. For sensory evaluation, only four antimicrobial additive treatments (control, PB+SL, SL+SDA, and PB+SL+SDA) are included. RTE turkey breast rolls were manufactured on a separate day and were sliced to 1.0 cm- thick pieces, and then vacuum-packaged.

Inoculation of test samples

The sliced turkey breast rolls (2-mm-thick) were transferred to the microbiology lab and were aseptically removed from the original bulk package into nylon-polyethylene bags (3 mil standard barrier, O₂ < 0.6 cm³/100 in²/24 h at 38 °C; Koch Industries, Kansas City, MO), one slice per bag. Each slice of sample was surface inoculated with 0.1 ml *L. monocytogenes* cocktail to approximately 10⁶ CFU/cm² level. Inoculated turkey roll samples were manually mixed for 30 s to distribute the inoculum evenly, then vacuum sealed (Multivac A300/16, Sepp Haggenmuller KG, Wolfertschwenden, Germany), and kept refrigerated overnight prior to irradiation.
Irradiation

All samples were irradiated using a Linear Accelerator Facility (Circe IIIR; Thomson CSF Linac, Saint-Aubin, France) at Iowa State University. The vacuum-packaged inoculated samples of each additive treatment were divided randomly into five groups and irradiated at 0 (control), 1.0, 1.5, 2.0 or 2.5 kGy. Samples irradiated at 0, 1.0 and 2.0 kGy were stored at 4°C for up to 42 days. The number of *L. monocytogenes* survivors in inoculated samples receiving 0, 1.0, 1.5, 2.0 and 2.5 kGy irradiation were analyzed at a 7-day interval.

For sensory analysis, the vacuum-packaged RTE turkey breast rolls of each additive treatment were randomly divided into two groups and irradiated at 0 (control) or 1.0 kGy. Sensory analysis was conducted 7 days after irradiation.

Microbiological analysis

Each package was aseptically opened using an alcohol-sterilized scissors. One hundred milliliters of sterile 0.1% peptone was added to each meat sample (surface area ~100 cm²) followed by pummeling at medium speed for 1 min in a stomacher. Samples were serially diluted with 0.1% peptone water and surface-plated (0.1 ml) in duplicate on modified oxford (MOX) agar plates to enumerate *L. Monocytogenes*. Typical *Listeria* colonies on MOX plates were counted after 48 h incubation at 35 °C.

Calculation of radiation *D*_10*-values*

The *D*_10 value, radiation dose (kGy) that results in 90% reduction of viable cells, was determined by plotting the log number of survivors per cm² (*Log*₁₀ CFU/cm²) versus irradiation dose (kGy). Linear regression curves were generated with SAS software (SAS, 2000). The *D*_10-value was calculated as the reciprocal of the absolute value of the slope of the regression line (Mendonca et al., 2004).

Sensory evaluation

After irradiation, the sliced vacuum-packaged RTE turkey rolls (0 kGy and 1.0 kGy) were directly transferred to sensory evaluation lab at Iowa State University. Ten trained
panelists participated in the evaluation of the sensory attributes of RTE turkey rolls. During training, panelists were familiarized with the sensory terms, the tasting techniques, and the computer software scoring system. Samples were evaluated for turkey-roll-like aroma, off-aroma, turkey-roll-like flavor, off-flavor and saltiness. Testing was conducted in partitioned booths and under red fluorescent lights. A line scale (numerical value of 15 units) was used with descriptive anchors (none and high) at each end of the line. Data were collected by using a computerized sensory system (COMPUSENSE five, v 4.0, Compusense, Inc., Guelph, Ontario, Canada). Before presenting to sensory panelists, the samples were heated in a microwave oven to 60 °C and labeled with random three-digit codes. Two sessions were conducted. In each session, panelists received samples from each of the eight treatments, with serving orders randomized. The measurements made on a given treatment by each panelist in the two sessions were averaged and used in the statistical analysis.

**Statistical analysis**

Data were analyzed by the General Linear Model (GLM) of the Statistical Analysis System (SAS, 2000). The differences in the mean values were compared by the Tukey's multiple comparison (P < 0.05), and mean values and standard error of the means (SEM) were reported.

**RESULTS**

**Antimicrobials and irradiation on the survival and growth of L. monocytogenes**

The initial pH value of turkey breast rolls without antimicrobials was around 6.38 (Table 1), which is similar to commercial RTE turkey breast rolls (unpublished data). Adding SDA in formulation slightly lowered the pH value of products, but the difference was very small. Adding PB in formulation did not change pH value of products. The pH of breast rolls of all treatments remained constant (change < 0.03) during 42 d storage (data not shown). The total plate counts for uninoculated samples at 0 day beyond detectible level in all six treatments (data not shown).
The survival curves of *L. monocytogenes* in RTE breast rolls with or without different antimicrobials following 0, 1.0, 1.5, 2.0, or 2.5 kGy irradiation were quite similar (Fig. 1), indicating that antimicrobial additives did not increase the irradiation sensitivity of *L. monocytogenes* in RTE turkey rolls. The $D_{10}$ value for breast rolls with various additive treatments ranged from 0.56 to 0.58 kGy.

Fig. 2 showed the growth of *L. monocytogenes* in non-irradiated vacuum-packaged RTE turkey rolls formulated with or without antimicrobial additives during storage at 4 °C. In control turkey rolls without any preservatives, *L. monocytogenes* proliferated rapidly and reached peak number ($8.2 \log_{10} \text{CFU/cm}^2$) after 14 days of storage at 4°C. Adding PB (0.1%) or SL (2%) in turkey rolls formulation failed to prevent *L. monocytogenes* from growing during refrigerated storage (Fig. 2). In both PB and SL treatments, *L. monocytogenes* reached a peak number of 8.0 Log after 21 days of refrigerated storage. Turkey rolls added with two combined antimicrobials showed 14 days of lag phase before *L. monocytogenes* growth (Fig. 2). PB+SL combination was less effective than SL+SDA combination in inhibiting the growth of *L. monocytogenes* in turkey breast rolls. In PB + SL treatment, *L. monocytogenes* reached peak a number of 7.8 Log after 35 days of refrigerated storage, while 42 days storage for the breast rolls treated with SL+SDA (Fig. 2). Including three-antimicrobial combination (PB+SL+SDA) was slightly more effective than two antimicrobial combinations in inhibiting the growth of *L. monocytogenes*. There was about 21 days lag phase before *L. monocytogenes* start to grow (Fig. 2).

The control turkey rolls irradiated at 1.0 kGy or 2.0 kGy (Fig. 3 and 4) had a 7-day lag phase. After the lag phase, the survived pathogen bacterial started to proliferate in 1.0 kGy and 2.0 kGy irradiated control turkey rolls and reached the peak after 21 and 35 days of refrigerated storage, respectively, indicating that low-dose irradiation itself could not provide safety margin for RTE turkey rolls. As showed in Fig. 3 and 4, an extended lag phase was observed in turkey rolls formulated with antimicrobial and irradiated at 1.0 and 2.0 kGy, and stored at 4 °C, especially for those turkey rolls with two or three combined antimicrobials. In turkey rolls with single antimicrobial, 0.1% PB or 2% SL, there was a 14 lag period after receiving 1.0 kGy irradiation, and then *L. monocytogenes* started to grow and reached $8 \log_{10} \text{CFU/cm}^2$ after 42 days storage (Fig. 3). In 2.0 kGy-irradiated turkey rolls formulated
with 2% SL or 0.1% PB, *L. monocytogenes* started to grow after 21 days of lag phase, and reached 6 and 5 Log_{10} CFU/cm² at day 42, respectively (Fig. 4), which is about the level before irradiation.

In turkey rolls formulated with two combined antimicrobials, PB+SL and SL+SDA, 1.0 kGy-irradiation extended lag phase to 21 and 28 days, respectively (Fig. 3), and 2.0 kGy kept *L. monocytogenes* in lag phase throughout storage (Fig. 4). Fig. 3 showed that a dose of 1.0 kGy was effective in suppressing the growth of *L. monocytogenes* in turkey rolls with PB+SL or SL+SDA for about six weeks when stored at 4 °C. During 42 days of refrigerated storage, 2.0-kGy-irradiation was listeriostatic for turkey rolls with two antimicrobials combinations (Fig. 4). In turkey rolls with three combined antimicrobials, PB+SL+SDA, and 1.0 or 2.0 kGy irradiation, *L. monocytogenes* stayed in the lag phase and no growth was observed after irradiation during the whole 42 days of refrigerated storage (Fig. 3 and 4).

**Sensory evaluation of breast rolls with antimicrobials**

The data in this paper indicated that the addition of two or three antimicrobials in combine with 1.0 kGy irradiation could provide safety margin for RTE turkey rolls during refrigerated storage. Therefore, turkey rolls formulated with basic formula, PB+SL, SL+SDA and PB+SL+SDA receiving 0 or 1.0-kGy-irradiation were chosen for sensory analysis. Table 2 showed the effect of antimicrobials on the sensory characteristics of RTE turkey breasts receiving 1.0 kGy irradiation. According to the sensory panelists’ evaluation, 1.0 kGy irradiation has no significant side effects on the sensory characteristics of RTE turkey breast (Table 2). Among turkey rolls without irradiation, turkey rolls formulated with SL+SDA had less turkey roll aroma and flavor, and higher off-aroma and off-flavor than other treatments (Table 2), but for 1.0 kGy irradiated RTE turkey breast rolls there was no significant difference in sensory characteristics between turkey roll with SL+SDA and without.

**DISCUSSION**

In RTE turkey rolls, adding antimicrobials did not affect the irradiation sensitivity of *L. monocytogenes*, which is different from that of turkey hams and bolognas. Including SDA in
formulation increased irradiation sensitivity of cured products such as RTE turkey ham and bologna (Sommer et al., 2003; Zhu et al., 2004c). The $D_{10}$ value obtained in this study was higher than the results from RTE turkey hams, where the same $L. monocytogenes$ cocktail were inoculated in which $D_{10}$ ranged from 0.48 to 0.52 kGy (Zhu et al., 2004c). This difference could be related to the nitrite added in ham. These $D_{10}$-values were higher than that of commercial frankfurters, bologna, turkey ham, which averaged about 0.44 kGy (Foong et al., 2004). The difference in $D_{10}$ value could be associated with the difference in formulation, $L. monocytogenes$ strains, the physiological state of the strain used, packaging condition, and plating medium (Patterson, 1989; Augustin, 1996; Tarte et al., 1996; Gürsel and Gürakan, 1997; Mendonca et al., 2004). In general, cells under stress showed higher levels of resistance to irradiation (Verma and Singh, 2001; Mendonca et al., 2004).

Irradiation resulted in a lag phase of the growth of $L. monocytogenes$ in turkey rolls, which is consistent with former reports (Gursel and Gürakan, 1997; Fong et al., 2004; Zhu et al., 2004c). Addition of antimicrobials in turkey formulation greatly increased the lag phase at each irradiation dose, indicating that the injured bacterial cells need more time to repair irradiation-caused damage in the presence of antimicrobials.

Our results showed that including single antimicrobial additive (2% SL or 0.1% PB) in turkey roll formulation was not sufficient to inhibit the proliferation of $L. monocytogenes$ survived irradiation, which is consistent with previous results from turkey ham (Zhu et al., 2004c) and other reports (Schlyter et al., 1993). In turkey slurries, Schlyter et al. (1993) found that 2.5% lactate failed to suppress the growth of $L. monocytogenes$ at 4 °C. The anti-listeria activity of antimicrobials combination was well documented. Qvist et al. (1994) found that no growth occurred in samples formulated with 2% SL and 0.25% glucono-delta-lactone during 35 days of storage at 5 °C and 10 °C (Qvist et al., 1994). A mixture of 2.5% lactate and 0.25% acetate inhibited the growth of $L. monocytogenes$ in vacuum-packed sliced RTE ham for 5 weeks at 4 °C (Blom et al., 1997). On cured smoked wieners, adding $\geq 1\%$ SL plus $\geq 0.1\%$ SDA inhibited the growth of $L. monocytogenes$ for 60 days at 4.5 °C (Glass et al., 2002). Turkey rolls without irradiation, however, 2% SL plus 0.1% SDA or 2% SL plus 0.1% PB antimicrobials combination delayed the growth of $L. monocytogenes$ for about two weeks, and then pathogen organism start to grow again at a lower growth rate than that
in the control turkey rolls or turkey rolls formulated with single antimicrobial. Including three antimicrobials combination in turkey roll formulation suppressed *L. monocytogenes* from growth for about 21 days. Including two or three combinations of antimicrobials were very effective in control of the growth of *L. monocytogenes* in turkey rolls receiving 1.0 or 2.0 kGy irradiation, and this was consistent with turkey hams (Zhu et al., 2004c).

Sensory analysis indicated that turkey rolls formulated with SL+SDA had less turkey-roll-like aroma and flavor than others, but no difference in turkey-ham-like aroma and flavor was observed when they were added to turkey ham (Zhu et al., 2004c). This could be related to the masking effect of intensive ham flavor and aroma. This lower aroma and flavor could be associated with a lower pH in the SL+SDA adding turkey rolls. Williams and Phillips (1998) reported that the off-flavor caused by SL increased as the pH of meat decreased. One intriguing question is that SL+SDA in combination with PB had little effect on sensory characteristics. The reason could be due to the increased pH after adding PB. As shown in Table 1, there was a slight increase in pH for breast rolls containing PB plus SL and SDA than that reported for SL+SDA. If this is the case, increasing phosphate content in the formulation for breast rolls containing SL+SDA could be a solution to avoid the side effects of SL+SDA on sensory, since phosphate can increase the pH of meat products. RTE turkey breast rolls with PB+SL and PB+SL+SDA were acceptable in sensory characteristics as judged by sensory panelists.

From the microbiological safety point of view, both PB+SL and SL+ SDA antimicrobial treatments in combination with 1.0 kGy or 2.0 kGy irradiation were effective in controlling post-packaging contamination and proliferation of *L. monocytogenes*, with SL+SDA more effective than PB + SL. Regarding sensory characteristics, PB + SL is better than SL + SDA in no irradiation turkey rolls, but no significant difference was detected between turkey rolls with PB+SL and with SL+SDA received 1.0 kGy irradiation. However, the volatile analysis indicated that the turkey rolls added with PB in formulation produced a significant amount of benzene during 1.0 kGy or 2.0kGy irradiation (Zhu et al., 2004b). Due to negative health effects of benzene (Mehlman, 2002), PB is not a proper antimicrobial for products receiving irradiation, thus SL+SDS combination is a better choice to control *L. monocytogenes* contamination in turkey rolls receiving low-dose irradiation.
CONCLUSION

A combination of physical and chemical antimicrobial intervention offers better inhibitory effect on the growth of *L. monocytogenes* in RTE turkey breast than that of single intervention. During 42 days of refrigerated storage, 1.0 kGy irradiated turkey rolls with PB+SL+SDA, 2.0-kGy-irradiated turkey rolls with PB+SL or SL+SDA are listeriostatic. In turkey rolls with PB+SL or SL+SDA, 1.0 kGy-irradiation could suppress the growth of *L. monocytogenes* for six weeks at 4°C. From the microbiology safety point of view, both PB+SL and SL+SDA antimicrobial treatments combined with 1.0 kGy or 2.0 kGy irradiation are promising technology. Considering benzene production from turkey rolls containing PB, SL+SDA is more suitable than PB+SL to ensure the *L. monocytogenes* safety of RTE turkey rolls.

REFERENCES


Table 1. Formulation of oven-roast turkey breast rolls and pH in processed products

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Control</th>
<th>PB</th>
<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Salt</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Phosphate (Brifisol)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Transglutaminase</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium caseinate</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Dextrose</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Water</td>
<td>6.25</td>
<td>6.25</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Potassium benzoate</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>Sodium lactate</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Sodium diacetate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

| pH Breast rolls | 6.38 | 6.38 | 6.37 | 6.38 | 6.34 | 6.35 |

Control= basic formula, PB= including 0.1% potassium benzoate, SL= including 2% sodium lactate, PB+SL= including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA= including 2% sodium lactate and 0.1% sodium diacetate, PSS= including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Fig. 1 Survival of *L. monocytogenes* following irradiation in turkey rolls with or without antimicrobial additives

Control = basic formula, PB = including 0.1% potassium benzoate, SL = including 2% sodium lactate, PB+SL = including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Fig. 2 Growth of *L. monocytogenes* at 4°C in non-irradiated vacuum-packaged RTE turkey breast rolls with or without antimicrobial additives

Control = basic formula, PB = including 0.1% potassium benzoate, SL = including 2% sodium lactate, PB+SL = including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Fig. 3 Viability of *L. monocytogenes* at 4°C in 1.0 kGy irradiated vacuum-packaged RTE turkey breast rolls with or without antimicrobial additives

Control = basic formula, PB = including 0.1% potassium benzoate, SL = including 2% sodium lactate, PB+SL = including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Fig. 4 Viability of *L. monocytogenes* in 2.0 kGy irradiated vacuum-packaged RTE turkey breast rolls with or without antimicrobials during 4°C storage

Control = basic formula, PB = including 0.1% potassium benzoate, SL = including 2% sodium lactate, PB+SL = including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Table 2. Sensory characteristics of RTE turkey breast rolls with or without antimicrobials, receiving 0 or 1.0 kGy irradiation.

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>Control</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
<td>-------</td>
<td>--------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Turkey rolls aroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>4.157ab</td>
<td>3.551ab</td>
<td>2.999b</td>
<td>5.261a</td>
<td>0.509</td>
</tr>
<tr>
<td>1.0 kGy</td>
<td>4.346</td>
<td>3.753</td>
<td>3.576</td>
<td>3.739</td>
<td>0.594</td>
</tr>
<tr>
<td>SEM</td>
<td>0.555</td>
<td>0.485</td>
<td>0.470</td>
<td>0.678</td>
<td></td>
</tr>
<tr>
<td><strong>Off-aroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>4.064b</td>
<td>5.620ab</td>
<td>6.941a</td>
<td>4.140b</td>
<td>0.733</td>
</tr>
<tr>
<td>1.0 kGy</td>
<td>3.805</td>
<td>5.963</td>
<td>5.955</td>
<td>5.916</td>
<td>0.783</td>
</tr>
<tr>
<td>SEM</td>
<td>0.757</td>
<td>0.769</td>
<td>0.759</td>
<td>0.750</td>
<td></td>
</tr>
<tr>
<td><strong>Turkey rolls flavor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>5.027</td>
<td>4.170</td>
<td>3.605</td>
<td>5.572</td>
<td>0.622</td>
</tr>
<tr>
<td>1.0 kGy</td>
<td>5.085</td>
<td>3.809</td>
<td>3.585</td>
<td>3.952</td>
<td>0.505</td>
</tr>
<tr>
<td>SEM</td>
<td>0.566</td>
<td>0.473</td>
<td>0.531</td>
<td>0.677</td>
<td></td>
</tr>
<tr>
<td><strong>Off-flavor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>3.424b</td>
<td>4.975ab</td>
<td>6.450a</td>
<td>3.641b</td>
<td>0.753</td>
</tr>
<tr>
<td>1.0 kGy</td>
<td>3.855</td>
<td>5.183</td>
<td>5.707</td>
<td>5.368</td>
<td>0.777</td>
</tr>
<tr>
<td>SEM</td>
<td>0.778</td>
<td>0.753</td>
<td>0.852</td>
<td>0.717</td>
<td>0.800</td>
</tr>
<tr>
<td><strong>Saltiness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>5.215</td>
<td>6.648</td>
<td>7.346</td>
<td>7.398</td>
<td>0.744</td>
</tr>
<tr>
<td>1.0 kGy</td>
<td>5.642</td>
<td>7.803</td>
<td>7.350</td>
<td>7.001</td>
<td>0.635</td>
</tr>
<tr>
<td>SEM</td>
<td>0.669</td>
<td>0.739</td>
<td>0.692</td>
<td>0.663</td>
<td></td>
</tr>
</tbody>
</table>

Means within a row with no common letter (a, b) differ significantly (P < 0.05).
Control = basic formula, PB+SL = including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
CHAPTER 9. ANTIMICROBIALS AND IRRADIATION ON THE SURVIVAL AND GROWTH OF LISTERIA MONOCYTOGENES AND QUALITY OF READY-TO-EAT TURKEY HAM

A paper submitted to Poultry Science


Abstract

Irradiation is an effective technology in eliminating L. monocytogenes but induces quality changes in meat products. To minimize irradiation-induced quality changes, only low irradiation doses are recommended. However, low-dose irradiation provides a chance for some pathogens to survive and proliferate during prolonged storage. To solve this problem, antimicrobials [2% sodium lactate (SL), 0.1% sodium diacetate (SDA), 0.1% potassium benzoate (PB)] in combined with low-dose irradiation were tested for their ability to inhibit the growth of L. monocytogenes during 4 °C storage and their effect on meat quality. The log_{10} reductions of L. monocytogenes in hams following 1.0 to 2.5 kGy irradiation ranged from 2.0 to 5.0. The D_{10} values were 0.52 kGy for control ham, ham with PB, SL or PB+SL, 0.49 kGy for ham with SL+SDA, and 0.48 kGy for ham with PB+SL+SDA. For hams formulated with SL+SDA or PB+SL, 1.0 kGy irradiation was effective in suppressing the growth of L. monocytogenes for about six weeks when stored at 4 °C, and 2.0-kGy-irradiation was listeriostatic. For hams with PB+SL+SDA, 1.0kGy was listeriostatic throughout storage. However, SL increased firmness of turkey hams and sensory panelists noted that the saltiness was a little higher in products containing SL, but the overall impact on quality was minimal. Significant amount of benzene was detected in irradiated hams with PB, showing

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PB was not fit as an antimicrobial for irradiated foods. In conclusion, 2% sodium lactate and 0.1% sodium diacetate in combination with low dose irradiation were effective in ensuring the safety of RTE meat products against *L. monocytogenes*.

**Keywords:** *Listeria monocytogenes*; e-beam irradiation; sodium lactate; sodium diacetate; potassium benzoate; RTE turkey ham.

**Introduction**

Ready-to-eat (RTE) meat products are the most popular meat products in the U.S. However, RTE meat products are frequently contaminated with *Listeria monocytogenes* mostly due to post-processing contamination. Of the random FSIS samples collected and analyzed between Jan. 1 and Sept. 30, 2003, 0.75 percent of RTE meats tested were positive for *L. monocytogenes* (Food Safety and Inspection Service, 2003). Due to its ability to grow at refrigerated temperature and its resistance to salt and nitrite (Tompkin, 2002; Lou and Yousef, 1999), any contaminated *L. monocytogenes* in cured or non-cured RTE meat products, which usually have long shelf-life and consumed directly without further heating, could proliferate to a threatening level during refrigerated storage. Because of its high mortality rate (~ 25%, Mead *et al.*, 1999) and economic impact due to products recall (Food Safety and Inspection Service released data, Oct. 17, 2003), *L. monocytogenes* is still a major food safety issue for processed meat industry. Currently, the U.S. Department of Agriculture (USDA) established a “zero tolerance” policy for *L. monocytogenes* in RTE meat products. The ubiquitous nature of *L. monocytogenes* (Beresford *et al.*, 2001) and its ability to grow at refrigerated temperature makes thermal processing and refrigerated storage are insufficient safety margin for processed meat products when “zero tolerance” are required. To ensure microbiological safety, apparently additional hurdles are needed. The post-package decontamination and formulating meat products with antimicrobial additives are common approaches to control *L. monocytogenes* post-processing contamination (Bedie *et al.*, 2001; Muriana *et al.*, 2002; Samelis, *et al.*, 2002).

Irradiation, one of the post-package decontamination technologies, is an effective way to destroy vegetative foodborne pathogens, including *L. monocytogenes* (Patterson *et al.*, 1993; Thayer, 1995; Thayer and Boyd, 2000). Significant amount of researches have been
conducted research on the effect of gamma irradiation in reducing *L. monocytogenes* in meat products (Patterson et al., 1993; Gürsel and Gürakan, 1997; Thayer, 1995; Thayer et al., 1998; Thayer and Boyd, 2000; Sommers et al., 2001; 2003). Gamma irradiation requires a radioactive source that makes it relatively unsafe and inconvenient to use. Electron beam (e-beam) irradiation uses accelerated electrons generated by high energy electrical power and is easier to control and safer to use than gamma irradiation. However, only few studies were conducted on the effectiveness of e-beam irradiation in eliminating *L. monocytogenes* in RTE meats (Foong et al., 2004).

Although effective in controlling microorganisms, irradiation negatively affects the quality of RTE meat even at 2.0 kGy (Zhu et al., 2003; 2004a). Because of this, only low-dosage irradiation would be practiced by meat industry in order to minimize quality changes. However some pathogens can survive low-dose irradiation and proliferated in RTE meats during storage (Sommers et al., 2003; Foong et al., 2004). Thus, it is necessary to use antimicrobials as an additional hurdle to curb the growth of pathogenic organisms that survived low dose irradiation. The antimicrobial activities of salts of organic acids such as lactate, acetate and diacetate are well documented (Blom et al., 1997, Bedie et al., 2001; Stekelenburg & Kant-Muermans, 2001; Glass et al., 2002; Mbandi & Shelef, 2002; Samelis, et al., 2002;), but there is limited information on the effectiveness of antimicrobials combined with irradiation in inhibiting the growth of *L. monocytogenes* (Sommers et al., 2003) in RTE meat products. In this study, 0.1% potassium benzoate (PB), 2% sodium lactate (SL) and 0.1% sodium diacetate (SDA) combinations were used as antimicrobials in turkey ham formulation. The effect of irradiation in combination with antimicrobials on the survival and growth of *L. monocytogenes* in RTE turkey hams during refrigerated storage were evaluated. The effects of antimicrobial additives and irradiation on the organoleptic quality of RTE turkey hams were also examined to assess the feasibility of using antimicrobials and irradiation as hurdles to ensure *L. monocytogenes* safety in RTE meats.

**Materials and Methods**

**Bacterial strains and growth conditions**
Five different *L. monocytogenes* strains (Scott A, H7969, H7596, H7762 and H7962) were used in this experiment. Prior to inoculation, each stock culture was individually grown in 10 ml Tryptic soy broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.6% yeast extract (Difco) (TSBYE) at 35 °C for 18 h. Then 1 ml of each strain was transferred individually to 100 ml of TSBYE and incubating at 35 °C for another 18 h. Each strain was harvested, washed twice, and re-suspended in sterile 0.1% (W/V) peptone (Difco) water. The inoculation cocktail was prepared by mixing equal volumes of the five strains suspension, which has approximately the same number of bacterial population.

**Preparation of RTE turkey meat products**

Oven roast turkey hams with different antimicrobial additives were freshly processed in the meat Lab at Iowa State University. Six antimicrobial additive treatments that include basic formula without any preservatives (control), with 0.1% potassium benzoate (PB), with 2% sodium lactate (SL), with 0.1% potassium benzoate and 2% sodium lactate (PB+SL), with 2% sodium lactate and 0.1% sodium diacetate (SL+SDA), or with 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate (PB+SL+SDA) were mixed with meat and other ingredients and then stuffed into large fibrous casings (φ=11.5cm). The rolls were heat processed to 74 °C internal temperature in an 84 C smoke house, chilled (4°C), and sliced to a thickness of 2 mm for microbiological measurements. For volatile, texture analysis and sensory evaluation, RTE turkey hams were sliced to 2.0 cm- thickness, and then vacuum packaged individually.

**Inoculation of test samples**

The sliced turkey ham (2-mm-thick) were transferred to the microbiology lab and were aseptically removed from the original bulk package into nylon-polyethylene bags (3 mil standard barrier, O₂ < 0.6cm³/100 in²/24 h at 38 °C; Koch Industries, Kansas City, MO), one slice per bag. Each slice of sample was surface inoculated with 0.1 ml *L. monocytogenes* cocktail to a level approximately 10⁶ CFU/cm². Inoculated turkey ham samples were manually mixed for 30 s to distribute the inoculum evenly, then vacuum sealed (Multivac A300/16, Sepp Haggenmuller KG, Wolfertschwenden, Germany), and kept refrigerated overnight prior to irradiation.
**Irradiation**

All samples were irradiated using a Linear Accelerator Facility (Circe IIIR; Thomson CSF Linac, Saint-Aubin, France) at Iowa State University.

The vacuum-packaged inoculated samples of each additive treatment were divided randomly into five groups and irradiated at 0 (control), 1.0, 1.5, 2.0 and 2.5 kGy. Samples irradiated at 0, 1.0 and 2.0 kGy were stored at 4°C for up to 42 days. The number of *L. monocytogenes* survivors in inoculated samples receiving 0, 1.0, 1.5, 2.0 and 2.5 kGy irradiation were analyzed at a 7-day interval.

For quality analysis, the vacuum-packaged RTE turkey hams of each additive treatment were randomly divided into three groups and irradiated at 0, 1.0, or 2.0 kGy. Volatile analysis was conducted at 0 and 28 days; texture and sensory analysis were conducted 7 days after irradiation.

**Microbiological analysis**

Each package was aseptically opened using an alcohol-sterilized scissors. One hundred milliliters of sterile 0.1% peptone was added to each meat sample (surface area ~100 cm²) followed by pummeling at medium speed for 1 min in a stomacher. Samples were serially diluted with 0.1% peptone water and surface-plated (0.1 ml) in duplicate on modified oxford (MOX) agar plates to enumerate *L. Monocytogenes*. Typical *Listeria* colonies on MOX plates were counted after 48 h incubation at 35°C.

**Calculation of radiation *D₁₀*-values**

The *D₁₀* value, radiation dose (kGy) that results in 90% reduction of viable cells, was determined by plotting the log number of survivors per cm² (*Log₁₀* CFU/cm²) versus irradiation dose (kGy). Linear regression curves were generated with SAS software (SAS, 2000). The *D₁₀*-value was calculated as the reciprocal of the absolute value of the slope of the regression line (Mendonca et al., 2004).

**Volatile analysis**
Volatiles of samples were analyzed using a Solatek 72 Multimatrix-Vial Autosampler/ Sample Concentrator 3100 (Tekmar-Dohrmann, Cincinnati, OH, USA) connected to a GC/MS (Model 6890/5973; Hewlett-Packard Co., Wilmington, DE, USA) according to the method of Ahn and Lee (2002). Two grams of minced turkey ham was placed in a 40-mL sample vial then put in a refrigerated (4 °C) sample tray. Samples were heated to 40 °C and purged with helium gas (40 mL/min) for 11 min. Volatiles were trapped with a Tenax/charcoal/silica trap column at 20 °C, desorbed for 2 min at 220 °C, concentrated using a cryofocusing unit at -90 °C, then desorbed into a GC column for 30 s at 220 °C. An HP-624 column (15 m, 250 μm i.d., 1.4 μm nominal), an HP-1 column (60 m, 250 μm i.d., 0.25 μm nominal), and an HP-Wax column (7.5 m, 250 μm i.d., 0.25 μm nominal) were combined using zero-volume connectors and used for volatile analysis. A mass selective detector (MSD) was used to identify and quantify volatile compounds in irradiated samples. The peak area was reported as the amount of volatiles released.

Sensory evaluation

After irradiation, the sliced vacuum packaged RTE turkey hams (0 kGy and 1.0 kGy) were directly transferred to the sensory evaluation lab at Iowa State University. Ten trained panelists participated in the evaluation of the sensory attributes of RTE turkey hams. During training, panelists were familiarized with the sensory terms, the tasting techniques, and the computer software scoring system. Samples were evaluated for turkey-ham-like aroma, off-aroma, turkey-ham-like flavor, off-flavor and saltiness. Testing was conducted in partitioned booths and under red fluorescent lights. A line scale (numerical value of 15 units) was used with descriptive anchors (none and high) at each end of the line. Data were collected by using a computerized sensory system (COMPUSENSE five, v 4.0, Compusense, Inc., Guelph, Ontario, Canada N1H 3N4). Before presenting to sensory panelists, the samples were heated in a microwave oven to 60°C and labeled with random three-digit codes. Two sessions were conducted. In each session, panelists received samples from each of the eight treatments, with serving orders randomized. The measurements made on a given treatment by each panelist in the two sessions were averaged and used in the statistical analysis.
Texture profile analysis

The RTE turkey ham were immobilized between specially constructed stainless steel plates, a star-shaped, cheery-pitter probe (Texture Technology Corp., New York, NY, USA) to penetrate the slices perpendicularly. Each sample underwent 2 cycles of 50% compression using the above probe fitted to a TA-XT2i® Texture Analyzer (Texture Technology Corp.). Two separate texture profile analyses (TPA) were done per slice, and 4 slices were used for each treatment. Five textural parameters, hardness, cohesiveness, springiness, chewiness, and resilience, were obtained from the force-time curve and calculated as described by Bourne (1978).

Statistical analysis

Data were analyzed by the General Linear Model (GLM) of Statistical Analysis System (SAS, 2000). The differences in the mean values of volatiles, sensory and texture scores were compared by the Tukey’s multiple comparison (P<0.05), and mean values and standard error of mean (SEM) were reported.

Results and Discussion

Irradiation sensitivity of Listeria monocytogenes

Fig. 1 showed survival curves of five-strain L. monocytogenes cocktail in turkey hams with single or combined antimicrobials following e-beam irradiation. Log_{10} reductions of L. monocytogenes in hams following 1.0 to 2.5 kGy irradiation ranged from 2.0 to 5.0. L. monocytogenes were slightly more sensitive to irradiation when combined antimicrobials were presented (Fig. 1).

Using linear regression of log_{10}CFU/cm² reduction following e-beam irradiation, the calculated D_{10}-value for control ham, hams containing PB, SL and PB+SL was about 0.52 kGy, while D_{10}-values for the hams containing SL+SDA and PB+SL+SDA were approximately 0.49 and 0.48, respectively, which is consistent with previous reports. Foong et al (2004) reported at there is no apparent difference in the irradiation D_{10}-values of smoked turkey with lactate and without lactate. Sommers et al. (2003) reported that adding sodium diacetate and potassium lactate (PL) increased the sensitivity of L. monocytogenes to gamma
irradiation. The $D_{10}$-value was 0.56 kGy for bologna without SDA and PL, 0.53 kGy for bologna containing 0.07% SDA-1% PL, and 0.46 kGy for bologna containing 0.15% SDA-2% PL (Sommers et al., 2003). The $D_{10}$-values in this study were higher than previously reported by Foong et al. (2004). They reported that the $D_{10}$-values for commercial frankfurters, bologna, turkey ham and roast beef ranged from 0.42 to 0.44 kGy, with an average of 0.44 kGy. That could be associated with the difference in RTE formulation, $L$. monocytogenes strains and plating medium (Tarte et al., 1996; Gürsel and Gürakan, 1997; Patterson, 1989).

**Effect of antimicrobials on the growth of L. monocytogenes survived irradiation**

Fig. 2a depicts the growth of $L$. monocytogenes in non-irradiated hams with single or combined antimicrobials during refrigerated storage (4 °C). Single or combined antimicrobials delayed the growth of $L$. monocytogenes in ham products. In control ham, $L$. monocytogenes reached the peak number after 21 days of refrigerated storage, while in hams containing two or three combined antimicrobials, $L$. monocytogenes increased less than one log after 42 days of refrigerated storage (Fig. 2a). For turkey ham, SL and SDA combination is more effective than PB and SL combination in inhibiting $L$. monocytogenes growth. The synergistic inhibitory effect of lactate and diacetate combination in inhibiting the growth of pathogenic organism has been documented (Bedie et al., 2001; Glass et al., 2002; Mbandi & Shelef, 2002; Samelis, et al., 2002; Stekelenburg, 2003). Samelis et al. (2002) reported that sodium lactate (1.8%; 3% of a 60% commercial solution) used in combination with 0.25% sodium acetate, sodium diacetate, or glucono-delta-lactone (GDL), sodium lactate inhibited the growth of $L$. monocytogenes in frankfurters during refrigerated storage. Cured smoked wieners formulated with combinations of $\geq1\%$ lactate plus $\geq0.1\%$ diacetate inhibited the growth of $L$. monocytogenes for 60 days at 4.5 °C (Glass et al., 2002). In turkey slurries formulated with 2.5% lactate and $\geq0.1\%$ diacetate, the growth of $L$. monocytogenes was inhibited for 42 days at 4 °C (Schlyter et al., 1993). Because inclusion of the high level (> 0.1%) of sodium diacetate in the formulation had a negative effect on the odor and taste of the final processed products (Stekelenburg & Kant-Muermans, 2001), only 0.1 % diacetate was included in the turkey ham formulation in this study.
As showed in Fig. 2b and 2c, at 1.0 and 2.0 kGy, an extended lag phase was observed in turkey hams stored at 4 °C, especially for those hams with combined antimicrobials. The increased lag phase was associated with irradiation dose, and addition of antimicrobials in turkey formulation greatly increased lag phase at each irradiation dose. During the lag phase, survived pathogens are believed to repair injuries caused by irradiation, and the organisms needed more time to repair irradiation damages when two or three antimicrobials were presented.

For control hams without antimicrobials, 1.0 kGy and 2.0 kGy delayed the lag phase for 7 and 14 days, respectively. This is consistent with the results of Foong et al. (2004) who reported irradiation of commercial turkey hams at 2.0 kGy and storing at 4 °C increased the lag phase of *L. monocytogenes* 2 weeks. For turkey hams formulated with two combined antimicrobials, 2% SL plus 0.1% SDA and 2% SL plus 0.1% PB, lag phase increased to 21 days in 1.0 kGy irradiated hams (Fig. 2b), and *L. monocytogenes* stayed in lag phase throughout storage in 2.0 kGy irradiated hams (Fig. 2c). In hams with three combined antimicrobials, 0.1% PB plus 2% SL plus 0.1% SDA, *L. monocytogenes* stayed in the lag phase during the refrigerated storage period in either 1.0 or 2.0 kGy irradiated hams (Fig. 2b, 2c).

After the lag phase, the survived pathogen bacterial started to proliferate in 1.0 kGy-irradiated control hams and reached a peak of 7.2 log<sub>10</sub> CFU/cm<sup>2</sup> after 28 days of refrigerated storage, which indicated that low dose, 1.0 kGy, irradiation itself could not provide safety margin for RTE turkey ham. Hams formulated with 2% SL+0.1% SDA and 0.1% PB+2% SL, a dose of 1.0 kGy was effective in suppressing the growth of *L. monocytogenes* for about six weeks when stored at 4 °C (Fig. 2b), and 2.0kGy irradiation is listeriostatic (Fig.2c). For hams with 0.1% PB+2% SL+0.1% SDA, both 1.0 kGy and 2.0 kGy are listeriostatic throughout storage (Fig. 2b, 2c). Sommers et al. (2003) reported that gamma radiation at a dose of 3.0 kGy prevented the proliferation of *L. monocytogenes* in bologna containing 0.07% SDA-1% PL and in bologna containing 0.15% SDA-2% PL over 8 weeks of storage at 9 °C.

*Effect of antimicrobial and irradiation on the quality of turkey hams*
The remaining question is the possible effect of antimicrobial combinations on the quality of irradiated turkey hams. To evaluate this, the volatiles, texture, and flavor of turkey hams were measured.

There were 43 volatiles identified by GC-MS. Since volatiles generated by irradiation have been extensively discussed in former publications (Du et al., 2002; Lee et al., 2003; Zhu et al., 2003), Table 2 only listed the amount of five selected representative volatiles. Irradiation increased hexane, 3-methyl butanal, 1-heptene, dimethyl disulfide and total volatile contents that is consistent with our former reports (Du et al., 2002; Lee et al., 2003).

Addition of antimicrobials largely had no effect on the volatiles of hams with or without irradiation, except for hams containing PB (Table 2). Irradiation greatly increased the amount of benzene detected in volatiles in hams containing PB. This is in agreement with our former report on turkey breast rolls, where significant amount of benzene was detected in products containing PB (Zhu et al., 2004b). Since benzene has negative effect on health (Mehlman 2002; Bogadi-Sare et al., 2003), PB+SL and PB+SL+SDA may not be good antimicrobial combinations for food that received irradiation despite their effectiveness in inhibiting the growth of *L. monocytogenes*.

The effect of antimicrobials on the organoleptic quality of hams, hams with antimicrobial additives receiving 0 and 1.0 kGy irradiation were evaluated by a trained sensory panel (Table 3). Microbial experiment showed that 1.0 kGy plus combined antimicrobial provided safety margin of turkey hams (Fig. 2b), thus 2.0 kGy irradiated samples are not included in sensory evaluation. For hams receiving 1.0 kGy irradiation or non-irradiated control, trained sensory panelists did not detect any significant differences in aroma, off-aroma, flavor and off-flavor. However, the saltiness of hams containing antimicrobials was significantly higher than that of control (ham without antimicrobial additives). This result indicated that salt level in formulation should be reduced slightly for hams adding antimicrobials. In a previous study on ground chicken, Jensen et al (2001) reported that 3% SL caused an increase in saltiness and bitterness that could be masked by the addition of 1% sucrose.

Table 4 depicted the texture profile data of turkey hams with/without antimicrobial additives before and after irradiation. For hams containing SL, the hardness is higher than the
rest of hams. The chewiness of products containing SL were also increased (Table 4). This should be due to the improved protein gelation during cooking, since 2% SL addition increased the ion content that is expected to improve protein solubility and water-binding capacity. Stekelenburg & Kant-Muermans (2001) reported that the firmness of the ham products formulated with 3.3% SL and 0.1% SDA was slightly tough, which is consistent with our results. If the increase in hardness, chewiness is undesirable in some products, slightly reducing phosphate or binders, such as transglutamase, in formulation should solve the problem (Lee and Park, 2003).

Conclusion

Irradiation is very effective in reducing L. monocytogenes in turkey hams, but survived L. monocytogenes could proliferate during subsequent storage. Antimicrobial combinations (PB+SL, SL+SDA and PB+SL+SDA) could be additional hurdles to low-dose irradiated RTE meat products to inhibit the growth of survived pathogens during prolong refrigerated storage. Sensory properties of turkey hams were not significantly affected by antimicrobial additives combined with 1.0 kGy irradiation. Because of a significant amount of benzene was detected in volatiles of hams containing PB, PB is not fit to be used as an antimicrobial additive in irradiated foods. Including 2% SL and 0.1% SDA in the formulation combined with 1.0 or 2.0 kGy irradiation is a promising technology to ensure the L. monocytogenes safety of RTE ham; at this concentration, these antimicrobials have no significant effect on meat quality.

References


Antimicrobials in the formulation to control Listeria monocytogenes postprocessing


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<th>SL+SDA</th>
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Control = basic formula, PB= including 0.1% potassium benzoate, SL= including 2% sodium lactate, PB+SL= including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA= including 2% sodium lactate and 0.1% sodium diacetate, PSS= including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Fig. 1 Survival of *L. monocytogenes* following irradiation in turkey hams with or without antimicrobial additives

Control= basic formula, PB= including 0.1% potassium benzoate, SL= including 2% sodium lactate, PB+SL= including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA= including 2% sodium lactate and 0.1% sodium diacetate, PSS= including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Fig. 2 Growth of *L. monocytogenes* on irradiated vacuum-packaged turkey ham during 4°C storage

a. 0 kGy; b. 1.0 kGy; c. 2.0 kGy

Control = basic formula, PB = including 0.1% potassium benzoate, SL = including 2% sodium lactate, PB+SL = including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA = including 2% sodium lactate and 0.1% sodium diacetate, PSS = including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Storage time (days)

- Control
- PB
- PB+SL
- PSS
- SL
- SL+SDA

Log$_{10}$ CFU/cm$^2$
Table 2. Selected volatiles of irradiated turkey hams with or without antimicrobial additives

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**Dimethyl Disulfide**

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**Total volatiles**

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^abc^ Means within a row with different letter (a, b, c) differ significantly (P < 0.05). N =4

^xyz^ Means within a column with different letter (x, y, z) differ significantly (P < 0.05).

Con = basic formula, PS = including 0.1% potassium benzoate and 2% sodium lactate, SS= including 2% sodium lactate and 0.1% sodium diacetate, PSS= including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Table 3. Sensory scores of turkey hams with different antimicrobial additives

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**Ham-like aroma**

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<td>4.1</td>
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</tr>
<tr>
<td>1.0 kGy</td>
<td>6.3</td>
<td>6.5</td>
<td>6.9</td>
<td>5.9</td>
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</tr>
<tr>
<td>SEM</td>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
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</table>

**Off- aroma**

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>Control</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
</tr>
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<tbody>
<tr>
<td>0 kGy</td>
<td>5.7</td>
<td>5.9</td>
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<td>0.6</td>
</tr>
<tr>
<td>1.0 kGy</td>
<td>4.6</td>
<td>4.7</td>
<td>4.1</td>
<td>4.8</td>
<td>0.6</td>
</tr>
<tr>
<td>SEM</td>
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**Ham-like flavor**

<table>
<thead>
<tr>
<th>Irradiation</th>
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<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kGy</td>
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<td>3.1</td>
<td>5.3</td>
<td>3.0</td>
<td>0.8</td>
</tr>
<tr>
<td>1.0 kGy</td>
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<td>3.8</td>
<td>5.7</td>
<td>4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>SEM</td>
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</table>

**Off- flavor**

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>Control</th>
<th>PB+SL</th>
<th>SL+SDA</th>
<th>PSS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kGy</td>
<td>5.5b</td>
<td>7.1ab</td>
<td>9.0a</td>
<td>6.8ab</td>
<td>0.6</td>
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<tr>
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<td>6.5ab</td>
<td>8.3a</td>
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<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

* a,b Means within a row with different letter (a, b) differ significantly ($P < 0.05$).
* x,y Means within a column with different letter (x, y) differ significantly ($P < 0.05$). N=10.
Control = basic formula, PB+SL = including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA= including 2% sodium lactate and 0.1% sodium diacetate, PSS= including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
Table 4. Texture analysis data for turkey hams with different antimicrobial additives

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>Control</th>
<th>PB</th>
<th>SL</th>
<th>PB+SL</th>
<th>SL+SDA</th>
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<th>SEM</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>2.031c</td>
<td>2.071bc</td>
<td>2.336&lt;sup&gt;abxy&lt;/sup&gt;</td>
<td>2.178&lt;sup&gt;bcx&lt;/sup&gt;</td>
<td>2.386ax</td>
<td>2.293abc</td>
<td>0.064</td>
</tr>
<tr>
<td>1.0 kGy</td>
<td>1.960b</td>
<td>2.184b</td>
<td>2.541ax</td>
<td>2.676ay</td>
<td>2.706y</td>
<td>2.489a</td>
<td>0.058</td>
</tr>
<tr>
<td>2.0 kGy</td>
<td>2.030c</td>
<td>2.057bc</td>
<td>2.224bcy</td>
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<td>0.050</td>
<td>0.061</td>
<td>0.060</td>
<td>0.072</td>
<td>0.054</td>
<td>0.061</td>
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</tr>
<tr>
<td>0 kGy</td>
<td>0.806b</td>
<td>0.816ab</td>
<td>0.823&lt;sup&gt;abxy&lt;/sup&gt;</td>
<td>0.832a</td>
<td>0.836axy</td>
<td>0.824ab</td>
<td>0.005</td>
</tr>
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<td>0.813bcx</td>
<td>0.831ab</td>
<td>0.821&lt;sup&gt;abx&lt;/sup&gt;</td>
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<td>0.005</td>
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<tr>
<td>2.0 kGy</td>
<td>0.798b</td>
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<td>0.840ay</td>
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<td>0.005</td>
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<tr>
<td>0 kGy</td>
<td>0.565a</td>
<td>0.572a</td>
<td>0.585a</td>
<td>0.567a</td>
<td>0.580ax</td>
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<tr>
<td>1.0 kGy</td>
<td>0.597a</td>
<td>0.580a</td>
<td>0.583a</td>
<td>0.581a</td>
<td>0.590axy</td>
<td>0.598a</td>
<td>0.007</td>
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<tr>
<td>2.0 kGy</td>
<td>0.588ab</td>
<td>0.593ab</td>
<td>0.607ab</td>
<td>0.580b</td>
<td>0.615ay</td>
<td>0.600ab</td>
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<tr>
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</tr>
<tr>
<td>0 kGy</td>
<td>0.925b</td>
<td>0.965b</td>
<td>1.124a</td>
<td>1.025abx</td>
<td>1.153ax</td>
<td>1.119ax</td>
<td>0.033</td>
</tr>
<tr>
<td>1.0 kGy</td>
<td>0.943b</td>
<td>1.032b</td>
<td>1.206a</td>
<td>1.289ay</td>
<td>1.309ay</td>
<td>1.242ay</td>
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<tr>
<td>2.0 kGy</td>
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<td>1.004bc</td>
<td>1.128ab</td>
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<td>1.190ax</td>
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<td>0.036</td>
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</tr>
<tr>
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<td>0.225a</td>
<td>0.235axy</td>
<td>0.220a</td>
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<tr>
<td>1.0 kGy</td>
<td>0.234a</td>
<td>0.221a</td>
<td>0.218ax</td>
<td>0.223a</td>
<td>0.225ax</td>
<td>0.238a</td>
<td>0.006</td>
</tr>
<tr>
<td>2.0 kGy</td>
<td>0.228a</td>
<td>0.231ab</td>
<td>0.243aby</td>
<td>0.226b</td>
<td>0.259ay</td>
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<td></td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>Means within a row with different letter (a, b, c) differ significantly (<i>P</i> < 0.05).

<sup>x,y,z</sup>Means within a column with different letter (x, y, z) differ significantly (<i>P</i> < 0.05). N=4.

Control= basic formula, PB= including 0.1% potassium benzoate, SL= including 2% sodium lactate, PB+SL= including 0.1% potassium benzoate and 2% sodium lactate, SL+SDA= including 2% sodium lactate and 0.1% sodium diacetate, PSS= including 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate.
CHAPTER 10. GENERAL CONCLUSION

*L. monocytogenes* is a big safety concern for RTE meat products, which are frequently contaminated by this pathogen. *L. monocytogenes* carried by live birds is one of the contamination sources. The effect of dietary vitamin E on the gut clearance of *L. monocytogenes* experimentally infected with *L. monocytogenes* in adult turkeys was studied. Results indicated that dietary vitamin E generally accelerated the clearance of inoculated *L. monocytogenes*. The accelerated *L. monocytogenes* clearance can boost the immune responses in turkeys receiving vitamin E supplementation. The CD4⁺, CD8⁺, as well as CD4⁺CD8⁺ T-lymphocytes were significantly higher in infected turkeys supplemented with vitamin E than those in infected turkeys with control diets. This result showed that vitamin E supplementation can be an approach to reduce *L. monocytogenes* contamination in live birds.

Irradiation is an effective post-packaging intervention technology to ensure microbiological safety of RTE meat products. Although quite a number of studies were conducted to determine the effect of gamma-irradiation on the survival of *L. monocytogenes*, little work have been done with e-beam irradiation in RTE meats, which was examined in this study. Irradiation (1.0 to 2.5 kGy) greatly reduced *L. monocytogenes* and aerobic plate counts in commercial RTE turkey ham and breast roll. The D₁₀ value of *L. monocytogenes* in breast roll and ham were 0.52 and 0.47 kGy, respectively, indicating that about 2.5 kGy are needed to achieve a 5-log reduction for *L. monocytogenes* in RTE turkey meat. Despite its effectiveness in eliminating pathogens and spoilage microorganisms, irradiation also causes quality changes. To evaluate this, turkey ham and breast rolls were irradiated at 1.0 or 2.0 kGy and stored at 4 °C for up to 14 days. Quality characteristics were analyzed weekly. Results showed that up to 2.0 kGy irradiation has limited effects on color and oxidation of vacuum-packaged commercial turkey ham, though a significant increase in redness was detected for turkey rolls. Irradiation has a significant influence on odor/flavor in vacuum-packaged turkey ham and breast rolls. Both sensory panelists and volatile analysis showed that there were significant changes in sulfur-related odor/flavor in RTE turkey products by irradiation. To avoid quality changes, therefore, only low-dose irradiation is recommended for RTE meat products. Unfortunately, some *L. monocytogenes* survives low-dose irradiation, and proliferates and causes a health hazard during refrigerated storage. Thus additional
barriers, such as adding preservatives, are necessary in order to ensure the microbial safety of products following low-dose irradiation.

A study was conducted to evaluate the effect of antimicrobials on the survival and growth of *L. monocytogenes* following electron-beam irradiation. Six antimicrobial additive treatments that include no preservatives (control), 0.1% potassium benzoate (PB), 2% sodium lactate (SL), 0.1% potassium benzoate plus 2% sodium lactate (PB+SL), 2% sodium lactate plus 0.1% sodium diacetate (SL+SDA), and 0.1% potassium benzoate, 2% sodium lactate and 0.1% sodium diacetate (PB+SL+SDA) were formulated into RTE turkey ham and breast rolls. Results showed that a combination of physical and chemical antimicrobial intervention offers better inhibitory effect on the growth of *L. monocytogenes* in RTE turkey meat than that of single intervention. A dose of 1.0 kGy was effective in suppressing the growth of *L. monocytogenes* in turkey rolls/hams formulated with PB+SL or SL+SDA for about six weeks when stored at 4 °C. No growth of *L. monocytogenes* after irradiation occurred during 42 days of storage for 2.0 kGy irradiated RTE turkey rolls/hams formulated with 0.1%PB+2%SL, 2%SL+0.1%SDA or 0.1%PB+2%SL+0.1%SDA, and 1.0 kGy irradiated turkey rolls/hams with 0.1% PB + 2% SL + 0.1% SDA. Thus, from the microbiology safety point of view, PB+SL and SL+SDA antimicrobial treatments combined with 1.0 kGy or 2.0 kGy irradiation were effective treatments to control post-packaging *L. monocytogenes* contamination in RTE turkey meats. The remaining question is the possible effects of antimicrobials combined with irradiation on the quality of RTE turkey meats. This information is needed to assess the feasibility of using antimicrobials and irradiation as hurdles to ensure *L. monocytogenes* safety in RTE meats. Results showed that 1.0 kGy irradiation has no significant effect on the sensory characteristics of RTE turkey meats. There is no significant difference in sensory characteristics among 1.0 kGy-irradiated RTE turkey meats with different antimicrobials. However, including 2% SL affected the texture and color values of turkey breast rolls. The addition of PB in RTE turkey meats produced a high amount of benzene after irradiation, suggesting benzoate salt is not a good antimicrobial to be used in products for irradiation. These results indicated that including 2% SL plus 0.1% SDA in formulation combined with 1.0 or 2.0 kGy irradiation is a promising technology to ensure the safety of RTE turkey rolls and hams from *L. monocytogenes*. 
ACKNOWLEDGEMENTS

First, I want to thank my major professor, Dr. Dong U. Ahn. He is really a great professor. During my Ph.D. study, we often discussed the experiments. Dr. Ahn always gave suggestions and then helped to arrange for the experiments. Besides experiments, Dr. Ahn also gave valuable suggestions related to my professional goals and personnel life. Thank you very much! Dr. Ahn.

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Finally, I want to give special thanks to my husband, Dr. Min Du, my son, Yilun Du, and my daughter Yiqing Du. Their love makes me feel energetic when tired, and it is this force that pushes me to go ahead! Every small progress I made has their contributions! I also want to thank my parents, my brother and sister, for every thing I achieved has their love and support associated with it.
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EDUCATION

2001/1 ~ Present: Department of Animal Science, Iowa State University
1995/9 ~ 1999/9: Plant Physiology and Molecular Biology/Biochemistry, College of Biological Sciences, China Agricultural University, Ph.D.
1991/9 ~ 1994/7: Biochemistry, College of Biological Sciences, China Agricultural University, M. Sc.
1987/9 ~ 1991/7: Biochemistry and Plant Physiology, College of Biological Sciences, China Agricultural University, B.Sc.

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2000/12~Present: Research Assistant, Department of Animal Science, Iowa State University
2000/2~2000/11: Post-doctorate Research Associate, Department of Zoology and Genetics, Iowa State University
1994/7 ~ 2000/1: Assistant Professor, Lecturer, Department of Plant Physiology and Biochemistry, China Agricultural University

RESEARCH EXPERIENCES

2001/12 ~ Present: The effects of irradiation and antimicrobial on the growth of Listeria monocytogenes and the meat quality of the ready to eat meat products
2000/12 ~2001/12: Rapid detection of Listeria contamination in animals by Taqman real-time PCR; influence of dietary vitamin E on the clearance of Listeria in turkeys experimentally infected with Listeria monocytogenes; lymphocyte population analysis with flow cytometry
2000/2~2000/11: Research on molecular mechanism of plant retrotransposons; cloning retrotransposons from wild plants by PCR, reconstructed into plasmids and transferred into tissue cultures

1995/9 ~ 2000/1: Construction of aquaporin cDNA expression vector; antibody preparation and Western Blot; RNA purification and Northern blot analysis; in situ hybridization analysis; analysis the function of aquaporin in plant cells by confocal microscopic observation


1995/1 ~ 1998/6: Cooperated research with the Department of Food Science in biochemical and molecular biological research of meat science.

RESEARCH GRANTS

1. National Natural Science Foundation of China (NSFC), Characterization of aquaporin in maize roots and its regulation in environmental stress, No. 39600096, 1997/1-1999/12

2. International Foundation for Science (IFS, Sweden, E2547-1) Biochemical changes in Jinhua ham processing and ways to accelerate, 1996/6-1998/6
   (Cooperated research with the Department of Food Science, China Agricultural University)

HONORS:

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Member of national honor society of agriculture, USA: GAMMA SIGMA DELTA.

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Midwest Poultry Consortium’s Center of Excellence, Summer Scholarship, 2003

Ned S. & Esther Raun graduate scholarship, 2003, Department of Animal Science, Iowa State University

Excellent young researcher award, 1998, China Agricultural University

Academic excellent award from the Ministry of Education, China, 1996

PUBLICATIONS


PRESENTATION IN THE MEETINGS


