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# **Control of Listeria** monocytogenes **Contamination in Ready-to-Eat Meat Products** Meijun Zhu, Min Du, Joseph Cordray, and Dong Uk Ahn

ABSTRACT: The ubiquitous nature of Listeria monocytogenes and its ability to grow at refrigerated temperature makes L. monocytogenes a significant threat to the safety of ready-to-eat (RTE) meat products. The contamination by L. monocytogenes in RTE meat primarily occurs during slicing and packaging after cooking. The effectiveness of postpackage decontamination technology such as in-package thermal pasteurization, irradiation, and high-pressure processing are discussed. Formulating meat products with antimicrobial additives is another common approach to control L. monocytogenes in RTE meat. Irradiation is an effective technology to eliminate L. monocytogenes but can influence the quality of RTE meat products significantly. The effect of irradiation or the combination of irradiation and antimicrobials on the survival of L. monocytogenes and the quality of RTE meat is discussed. Keywords: Listeria monocytogenes, ready-to-eat, meat, preservative, irradiation, quality

Introduction

Listeriosis accounts for about 2500 cases of illness and approximately \$200 million in monetary loss in the United States annually (CDC 2002). Ready-to-eat (RTE) cooked meats are frequently contaminated with *Listeria monocytogenes* during postprocessing steps (Beresford and others 2001). The contamination by L. monocytogenes of cured and non-cured RTE cooked meat is a major safety concern for RTE cooked meat products because (1) RTE cooked meats have long shelf-life and are consumed without further heating, (2) *L. monocytogenes* can proliferate to a threatening level during refrigerated storage because of its ability to grow in the presence of curing salt at refrigerated temperature (Lou and Yousef 1999), and (3) the emergence of multiple resistance in Listeria spp. due to acquisition of a replicon from staphylococci (Lemaitre and others 1998).

A cumulative report about *L. monocytogenes* contaminations in 9 different categories of RTE meat and poultry products between 1990 and 1999 listed 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-y L. monocytogenes prevalence for dry and semidry fermented sausages was 3.25% (Levine and others 2001) and the random Food Safety and Inspection Service (FSIS) samples of RTE meats collected and analyzed between Jan. 1 and Sept. 30, 2003,

were 0.75% (FSIS 2003a). A survey of L. monocytogenes contamination in RTE products was conducted at retail markets in Maryland and northern California. Of 31705 samples tested, 577 were positive. The overall prevalence was 1.82%, with prevalences ranging from 0.17% to 4.7% among the product categories, with in-store-packaged foods significantly higher than manufacturerpackaged foods (Gombas and others 2003).

The safety concern of *L. monocytogenes* is further highlighted by several well-publicized outbreaks of listeriosis involving RTE meat products. The Centers for Disease Control and Prevention (CDC 1999) reported that a multistate outbreak between 1998 and 1999, which caused 101 cases and 21 deaths, was linked to the contamination by L. monocytogenes in frankfurters and deli meats. In 2000, a multistate outbreak involving deli turkey meat resulted in 29 cases, 4 deaths, and 3 miscarriages or stillbirths (CDC 2000). More recently, a multistate outbreak of L. monocytogenes infections in the northeastern United States was attributed to the consumption of sliceable turkey deli meat. There were 46 confirmed cases, 7 deaths, and 3 stillbirths or miscarriages associated with this outbreak (CDC 2002). These reports clearly indicate that RTE meat products are associated with listeriosis. The recall of 26 million pounds of turkey meats in 2002 indicates the economic consequences of RTE meats contaminated with L. monocytogenes (USDHHS 2002). Currently, the U.S. Dept. of Agriculture (USDA) established a "zero tolerance" policy for L. monocytogenes in RTE meat products. Therefore, it is important to prevent the contamination of L. monocytogenes in RTE meat products.

#### Sources of L. monocytogenes Contamination

Listeria monocytogenes is a Gram-positive, non-sporeforming, highly mobile, rod-type, facultative anaerobic bacterium (Farber

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and Peterkin 1991). It can grow in a wide range of temperature conditions (0 °C 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 6.0 and poorly or not at all below pH 5.0 (Glass and Doyle 1989). It tolerates salt and nitrite (McClure and others 1997). It is widely present in plant, soil, silage, sewage, slaughterhouse waste, human and animal feces, processing environments, and catering facilities (Farber and Peterkin 1991; Beresford and others 2001). Feed may be an important source of contamination for animals (Fenlon and others 1996). Adult animals may be transiently colonized by consuming contaminated feed or water (Husu and others 1990). Thus, *L. monocytogenes* may enter the packing plant at low levels in the intestines of recently infected animals. Some L. monocytogenes strains may survive in bio?lms, persist in processing environments, and ultimately contribute to both environmental and RTE product contamination (Giovannacci and others 1999). Because L. monocytogenes is sensitive to heat treatment, it can easily be inactivated by cooking. Therefore, post-cooking recontamination during packaging is the main concern.

The processing facilities were frequently contaminated with nonpersistent or persistent *L. monocytogenes*, which play an important role in contaminating products. The contamination status of processing lines and machines was influenced by the compartmentalization of the processing line, with poor compartmentalization increasing *L. monocytogenes* contamination (Lunden and others 2003). *L. monocytogenes* has the ability to adhere to stainless steel, but significant differences exist in the ability of various *L. monocytogenes* strains to attach to a surface. Dust contaminated with *L. monocytogenes* was another source of contamination (De Roin and others 2003).

#### Intervention of L. monocytogenes Contamination

*L. monocytogenes* contaminated in meat could be virtually eliminated during the cooking step of RTE meats processing. Therefore, *L. monocytogenes* contamination in RTE meats is primarily due to post-cooking contamination. Post-package decontamination methods such as in-package thermal pasteurization and irradiation, and formulating meat products with antimicrobial additives are common approaches to control of *L. monocytogenes* in RTE meat.

Depending on whether there are post-lethality treatments and growth inhibitors, the susceptibility of RTE foods to *L. monocytogenes* contamination varies. To effectively control *L. monocytogenes* in RTE foods, the FSIS published a final rule that stipulates 3 alternatives:

Alternative 1—Use both a post-lethality treatment and a growth inhibitor for *Listeria* on RTE products. Establishments opting for this alternative will be subject to FSIS verification activity that focuses on the post-lethality treatment effectiveness. Sanitation is important but is built into the degree of lethality necessary for safety as delivered by the post-lethality treatment.

Alternative 2—Use either a post-lethality treatment or a growth inhibitor for *Listeria* on RTE products. Establishments opting for this alternative will be subject to more frequent FSIS verification activity than for Alternative 1.

Alternative 3—Use sanitation measures only. Establishments opting for this alternative will be targeted with the most frequent level of FSIS verification activity. Within this alternative, FSIS will place increased scrutiny on operations that produce hot dogs and deli meats. In a 2001 risk ranking, the FSIS and the Food and Drug Administration identified these products as posing a relative high risk for illness and death (FSIS 2003b). Therefore, it is to the manufacturer's advantage to take measurements for reducing *L. monocytogenes* contamination in food.

#### In-package thermal pasteurization

The effect of surface pasteurization temperatures on the survival of *L. monocytogenes* in low-fat turkey bologna showed that all the *L. monocytogenes* cells were destroyed after exposure to an 85 °C water bath for 10 s (>6 log reduction), but viable cells were detected at up to 10 min of heating at 61 °C (<6 log reduction). The D-values for *L. monocytogenes* at 61 °C and 65 °C were 124 s and 16.2 s, respectively (McCormick and others 2003). Muriana and others (2002) reported that submersion heating of RTE deli meats at 90.6 °C to 96.1 °C for  $\geq$  2 min could readily provide 2-log reductions. In beef, the D-values of *L. monocytogenes* at 60 °C (D60 °C), 65 °C, 71.1 °C, and 73.9 °C were 4.67, 0.72, 0.17, and 0.04 min, respectively (Juneja 2003). The effectiveness of inpackage pasteurization in inactivating pathogenic organisms depended upon package size and the roughness of the product surface (Muriana and others 2002; Murphy and others 2003b).

The strains of L. monocytogenes also influence the effectiveness of thermal pasteurization: in an open vessel, the D60 °C values of L. monocytogenes strains ranged from 1.3 to 6.5 min whereas D72 °C varied from 0.06 to 1.5 s in capillary tubes (Lemaire and others 1989). Adding 4.8% sodium lactate (SL) to beef increased heat resistance of L. monocytogenes. Sodium diacetate (SDA) (0.25%) interacted with SL and reduced the protective effect of SL, which rendered 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 increased the tolerance of cells to mild heat stress (56 °C to 62 °C) (Anderson and others 1991). Cells grown at 42.8 °C before heat treatment were more thermotolerant than those grown at 37 °C (Rowan and Anderson 1998). Heating at slowly increasing temperatures ( $\leq 0.7$  °C/min) enhanced the thermotolerance of L. monocytogenes (Stephens and others 1994), and starvation in phosphate-buffered saline pH 7 for 6 h at 30 °C increased the heat resistance of L. monocytogenes in broth but not in hot dog batter (Mazzotta and Gombas 2001).

#### Irradiation

lonizing radiation is a process in which products are exposed to radiant energy (Andrews and others 1998). lonizing radiation 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 (5.27 y and 30.1 y, respectively) and high penetration power and 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 penetrate only about 5 cm. X-irradiation, which has intermediate properties of the 2 previously discussed irradiation methods, penetrates foods more shallowly than gamma irradiation but much more deeply than electron beams (Sadler and others 2001).

E-beam irradiation was reported to be more effective than gamma-ray irradiation in decreasing *Bacillus cereus* and *Escherichia coli O157:H7*, but not for *L. monocytogenes* (Miyahara 2002). In cooked pork chops and hams inoculated with *L. monocytogenes*, low-dose (0.75 to 0.90 kGy) irradiation reduced *L. monocytogenes* by more than 2 log (Fu and others 1995). Shamsuzzaman and others (1995) reported that the combination of heating and ebeam irradiation was very effective in eliminating inoculated *L. monocytogenes* in chicken breast meat. Foong and others (2004) reported that the doses of e-beam irradiation 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. Clardy and others (2002) found that a dose of 3.5 to 4.0 kGy was needed to achieve a 5-log reduction of *L. monocytogenes*.

The resistance of *L. monocytogenes* to radiation varies depending on strains and the physiological state of the strain used (Augustin 1996; Tarte and others 1996). In general, cells under stress

show higher levels of resistance to irradiation (Verma and Singh 2001). Starved cells consistently exhibited higher irradiation  $D_{10}$ values than controls in both saline and ground pork (Mendonca and others 2004). Irradiation of ground pork at 2.5 kGy reduced L. monocytogenes without starvation by approximately 6.0 log, whereas starved cells were reduced by only 3.8 log (Mendonca and others 2004). The resistance of L. monocytogenes to radiation also depends on the substrate in which the organism grows (Augustin 1996). Thayer and others (1998) found that the doses of gamma-irradiation for *L. monocytogenes* inactivation were significantly different between raw and cooked nuggets. Sommers and others (2003a) reported that adding SDA and potassium lactate (PL) increased the sensitivity of *L. monocytogenes* to irradiation. The radiation doses required to eliminate 90% of the viable L. monocytogenes cells were 0.56 kGy for bologna containing 0% SDA-0% PL, 0.53 kGy for those containing 0.07% SDA-1% PL, and 0.46 kGy for those containing 0.15% SDA-2% PL (Sommers and others 2003a). Foong and others (2004) and Zhu and others (2005), however, reported that adding SL to the RTE meat formulation did not increase radiation sensitivity of L. monocytogenes to e-beam irradiation. Salt content in product also affects the effectiveness of irradiation in killing pathogenic organisms. Highly significant effects 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). The D-values for S. typhimurium were 0.48, 0.73, 0.78, 0.87, and 0.72 kGy in meat rehydrated with 0%, 25%, 50%, 75%, and 100% NaCl solution (Thayer and others 1995). Adding citric acid to frankfurters enhanced the lethality of ionizing radiation (Sommers and others 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). One concern about using modified atmosphere packaging in irradiated meat or poultry, however, is that pathogens may grow and/or produce toxins because of low competing organisms. This is of even greater concern if spoilage is suppressed and does not provide the usual warning signals (Lee and others 1996).

Temperature effects must be carefully considered since 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 to inactivate the foodborne pathogens (Thayer 1995). *E. coli* O157:H7 had a significantly higher  $D_{10}$  value when irradiated at -17 °C to -15 °C than when irradiated at 3 °C to 5 °C (Lopez-Gonzalez and others 1999). 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  $D_{10}$  values or higher resistance (Lopez-Gonzalez and others 1999).

All previously discussed results indicated that manufacturers should consider factors present in their products that may allow *L. monocytogenes* to resist irradiation. Generalization of the effects of irradiation may be misleading because the effectiveness of irradiation is affected by irradiation conditions and products composition.

#### **Food preservatives**

**Chemical antimicrobials**. The salt of lactate is frequently used as an antimicrobial in meat products due to its beneficial properties to meat quality when applied at appropriate concentrations. The addition of lactate to food products with neutral pH offers good prospects for shelf-life prolongation (Houtsma and others 1993). In general, Gram-positive bacteria were more sensitive toward lactate than Gram-negative bacteria under optimum growth conditions (pH 6.5, 20 °C) (Houtsma and others 1993). Recently, more attention was paid to the combined application of lactate and diacetate due to the synergistic inhibitory effect of lactate and diacetate in inhibiting the growth of pathogenic organism in meat products (Glass and others 2002; Mbandi and Shelef 2002; Samelis and others 2002; Stekelenburg 2003).

The growth of L. monocytogenes in cooked meat products could be suppressed by using suitable amounts of SL in combination with low pH: the growth of L. monocytogenes was effectively controlled in artificially contaminated vacuum-packaged sausages formulated with 1.8% to 2% SL and 0.25% sodium acetate, SDA, or glucono-delta-lactone during refrigerated storage (Qvist and others 1994; Blom and others 1997; Samelis and others 2002). In turkey slurries, Schlyter and others (1993) found that adding 2.5% lactate and 0.1% SDA combination prevented the growth of L. monocytogenes about 42 d at 4 °C, but 0.1% SDA itself was not effective. In cured smoked wieners, adding  $\geq 1\%$  SL plus  $\geq 0.1\%$ SDA inhibited the growth of L. monocytogenes for 60 d at 4.5 °C (Glass and others 2002). 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 d at 10 °C. At 5 °C, a listeriostatic effect was produced by 1.8% SL plus 0.1% SDA. Our results showed that in RTE turkey hams containing 2% SL plus 0.1% SDA or 2% SL plus 0.1% potassium benzoate (PB), L. monocytogenes increased less than 1 log during 42 d of refrigerated storage (Zhu and others 2005).

A chemically synthesized short-chain peptide composed of 6 leucine and 8 lysine residues was shown to be biocidal against several foodborne organisms including *L. monocytogenes* suspended in phosphate buffer at concentrations 5 to 50  $\mu$ g/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 *L. monocytogenes* in chicken meat, especially after several days of refrigerated storage (Capita and others 2001). Sodium hypochlorite, quaternary ammonium compound, and peroxyacetic acid are used as sanitizers in meat processing plants were effective in eliminating *L. monocytogenes* (Romanova and others 2002; Stopforth and others 2002).

Lactobacilli, probiotic bacteria, and bacteriocins. Biopreservation with various strains of lactic acid bacteria is a suitable alternative to chemical preservatives (Jacobsen and others 2003). The antimicrobial activity of a bacteriocin-producing *Lactobacillus plantarum* MCS strain against *L. monocytogenes* was observed in naturally and artificially contaminated salami (Campanini and others 1993). Further, *Pediococcus, Bifidobacteria*, and *Enterococcus* all showed strong antimicrobial activities toward *L. monocytogenes* (Baccus-Taylor and others 1993; Bevilacqua and others 2003; Leroy and others 2003). Thus, the application of *Lactobacillus, Pediococcus*, or *enterococci* bacteria in starter cultures may provide an additional hurdle against listeriosis in fermented meat products.

Lactic acid bacteria can also be used to inhibit the growth of *L.* monocytogenes in nonfermented products. Addition of *Lactobacillus sake* Lb 706 prevented the growth of *L.* monocytogenes in pasteurized minced meat and comminuted cured raw pork during the 1st few days after production (Schillinger and others 1991). The *Lactobacillus sakei* strain applied to cooked products at a concentration of 10<sup>5</sup> to 10<sup>6</sup> colony-forming units (CFU)/g immediately before slicing and vacuum-packaging inhibited the growth of a cocktail of 3 rifampicin-resistant mutant *L.* monocytogenes strains both at 8 °C and 4 °C (Bredholt and others 2001). A combined culture of lactic acid strains—*Pediococcus acidilactici, Lactobacillus casei,* and *Lactobacillus paracasei*—added to frankfurters and cooked ham coinoculated with *L.* monocytogenes showed bacteriostatic activity in cooked ham and bactericidal activity in frankfurters (Amezquita and Brashears 2002). Jacobsen and others (2003) reported that the live cells of bacteriocin-producing *Leuconostoc carnosum* 4010 inhibited the growth of *L. monocytogenes* in cooked, sliced, and gas-packed meat products stored at 5 °C and 10 °C for 4 wk.

The main reason for the effectiveness of protective bacteria in killing *L. monocytogenes* is due to the production of bacteriocin (Campanini and others 1993; Katla and others 2002; Jacobsen and others 2003). Bacteriocins are ribosomally synthesized polypeptides produced by bacteria with an ability to kill or inhibit the growth of similar bacterial strain(s). 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 and others 1996).

Considerable research has been carried out on the antilisterial properties of nisin in foods: beef cubes inoculated with approximately 7 log CFU/mL of L. monocytogenes Scott A and treated with nisin or nisin combined with ethylenediaminetetraacetic acid (EDTA) by dipping in these solutions for 10 min reduced the population of L. monocytogenes by 2.01 and 0.99 log CFU/cm<sup>2</sup> as compared with the control, respectively, under vacuum and storage at 4 °C for up to 30 d (Zhang and Mustapha 1999). Nisin at 400 IU/mL or 400 IU/mL of nisin in combination with 2% lowmolecular-weight polylactic acid or 2% lactic acid showed immediate bactericidal effects on L. monocytogenes Scott A in vacuumpackaged beef (Ariyapitipun and others 2000). Nisin incorporated into thermally compacted soy films reduced the cell number on turkey bologna from 10<sup>6</sup> to 10<sup>5</sup> after 21 d of storage (Dawson and others 2002). Packaging films coated with a cellulose-based solution containing 10000 and 7500 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 and others 2004).

Other bacteriocins also showed antilisterial activity: reuterin produced by Lactobacillus reuteri strain 12002 at a concentration of 250 activity units/g resulted in 3.0-log10 reduction of L. monocytogenes in raw ground pork after 1 wk of storage at 7 °C (El-Ziney and others 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  $\mu$ g/g) had a bacteriostatic effect throughout the storage period of 4 wk, whereas a low dosage (12 ng/g) permitted initial growth, but at a slower rate (Katla and others 2002). Enterocins, bacteriocins produced by enterococci, also hold considerable promise as alternatives to traditional chemical preservatives to control pathogens in meat products (Hugas and others 2003; Leroy and others 2003), and so was pediocin, a bacteriocin produced by Pediococcus (Degnan and others 1993; Garriga and others 2002).

However, current regulation is hampering the application of purified bacteriocins (Hugas and others 2003). Furthermore, bacteriocins are amphiphilic peptides susceptible to adsorption to food macromolecules and proteolytic degradation. Aasen and others (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. Less than 1% of the total bacteriocin activities were left after 1 wk in cold-smoked salmon and even less in raw chicken, whereas the activity was stable for more than 4 wk in cooked meat.

**Plant extracts.** Recently, much attention was paid to plant extracts due to their antioxidant and antimicrobial activities. 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) showed antibacterial activity to many pathogenic bacteria (Del Campo and

others 2000). Antibacterial activity of the rosemary extract was strongly influenced by the composition of media and increased by low pH, high NaCl contents, and low temperatures. However, lipids, surface-active agents, and some proteins decreased its antibacterial activity (Del Campo and others 2000).

Many other plant extracts such as the essential oil from Thymus eigii, Picea excelsa, and Camellia japonica L. showed antibacterial activities against pathogenic bacteria (Kim and others 2001; Canillac and Mourey 2004; Tepe and others 2004). Hao and others (1998) found that eugenol (clove extract) and pimento extract significantly inhibited the growth of Aeromonas hydrophila and L. monocytogenes inoculated in cooked beef slices. Larson and others (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 in treated raw ground beef declined when 1% pine bark extract was used (Pycnogenol), grape seed extract ActiVin, or rosemary oleoresin after 9 d of refrigerated storage. The results suggested that these natural extracts had potential to be used with other preservation methods to reduce pathogens in ground beef (Ahn and others 2004).

#### **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. Thus, HPP can inactivate microorganisms without significant changes in texture, color, or nutritional value of food (Hugas and others 2002; Ross and others 2003). HPP is not only a powerful tool to control pathogenic organisms but also is effective to spores and viruses. Generally, Gram-positive organisms are more recalcitrant to pressure inactivation than Gram-negative bacteria (Hugas and others 2002; Solomon and Hoover 2004). Mussa and others (1999) reported that L. monocytogenes inoculated to packaged fresh pork was more resistant to pressure inactivation than indigenous microflora in fresh pork. Among 9 tested L. monocytogenes strains, significant variability was observed in response to HPP (Tay and others 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 and others 2002). Microbial inactivation was increased with prolonged exposure to pressure or increased pressure (Simpson and Gilmour 1997; Ponce and others 1998; Lucore and others 2000). Pressurization at 700 MPa showed quick inactivation of L. monocytogenes (Lucore and others 2000). HPP with 250 MPa did not inactivate L. monocytogenes, but significant lag phases of 17 and 10 d were observed at 5 °C and 10 °C, respectively (Lakshmanan and Dalgaard 2004). Temperature influences the effectiveness of microbial inactivation by HPP (Hugas and others 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 and others 1998). Ananth and others (1998) also showed that the effectiveness of HPP was slightly reduced at room temperature compared with refrigerated temperature. The presence of oil reduced the effectiveness of high pressure in killing L. monocytogenes (Simpson and Gilmour 1997). Cell morphology also had an effect on HPP, with bacilli being more sensitive to pressurization than cocci (Hugas and others 2002). When HPP was combined with antimicrobials, like bacteriocins, the death rate increased because of sub-lethal injuries to living cells (Garriga and others 2002).

#### **Hurdle technology**

The concept of hurdle 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. A synergistic effect could be achieved if the hurdles hit at the same time at 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. However, these hurdles seem insufficient when it comes to L. monocytogenes due to its ubiquitous nature (Beresford and others 2001), ability to grow at refrigerated temperature and anaerobic condition, and resistance to salt and nitrite (Lou and Yousef 1999). Postprocessing contamination of RTE meat with L. monocytogenes during slicing and packaging is difficult to avoid. To ensure microbiological safety of RTE meats, therefore, additional hurdles are needed. Formulating meat products with antimicrobial additives are common practice to control the growth of L. monocytogenes after processing (Glass and others 2002; Mbandi and Shelef 2002; Samelis and others 2002; Stekelenburg 2003). Post-package decontamination such as post-package heating (Muriana and others 2002; Murphy and others 2003a, 2003b), irradiation (Fu and others 1995; Thayer and Boyd 1999; Foong and others 2004), and HPP (Ananth and others 1998; Lucore and others 2000; Tay and others 2003) are additional hurdles for RTE meats.

Irradiation is very effective in eliminating contaminated L. monocytogenes. However, pathogens that survived irradiation could grow and proliferate during refrigerated storage (Foong and others 2004; Zhu and others 2005), and thus additional hurdles are needed. Antimicrobials were used in combination with irradiation to suppress the growth of *L. monocytogenes* after irradiation. Irradiation of L. monocytogenes suspended in SDA resulted in synergistic reductions of the microorganism. SDA can inhibit the proliferation of *L. monocytogenes* surviving the irradiation process with minimal impact on the color, lipid oxidation, and firmness of fine-emulsion sausage when used within regulatory limits (Sommers and Fan 2003). Turkey hams formulated with 2% SL + 0.1% SDA and 0.1% PB + 2% SL in combination with 1.0 kGy irradiation was effective in suppressing the growth of L. monocytogenes for about 6 wk at 4 °C, and 2.0 kGy irradiation was listeriastat (Zhu and others 2005). Adding bacteriocins such as nisin and pediocin AcH into formulation is another choice. Including bacteriocins into meat formulation increased the pathogen death rate during high-pressure processing (Garriga and others 2002).

#### Irradiation and Quality of Meat Products

### Irradiation odor

Although irradiation is very effective in controlling food-borne pathogens in meat, it generates free radicals that cause lipid peroxidation and other chemical changes and influences the quality of meat (Hashim and others 1995; Patterson and Stenenson 1995; Ahn and others 1998; Ahn and Lee 2004). Irradiated meat products can develop a characteristic odor described as "bloody sweet" or "barbecued corn-like" (Hashim and others 1995; Ahn and Lee 2004). Patterson and Stevenson (1995) reported that dimethyl trisulphide was the most potent and obnoxious volatile compound from irradiated raw chicken, followed by cis-3, trans-6 nonenals, 1-octenone and bis (methylthio-) methane. More research indicated that the volatiles responsible for the off-odor in irradiated meat were sulfur-containing compounds such as methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide (Ahn and others 2000a, 2000b; Fan and others 2002; Nam and Ahn 2003a; Zhu and others 2003). Ahn and others (2000a, 2000b) and Fan and others (2002) reported that dimethyl disulfide and other sulfur compounds increased dramatically after irradiation. Sensory analysis indicated that sulfur odor increased as irradiation dose increased (Zhu and others 2003). In addition to sulfur compounds, irradiation dramatically increased 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 and others 2000a, 2000b; Jo and Ahn 2000).

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

Irradiating various amino acid homopolymers produced different odor characteristics, but the majority of newly generated and increased volatiles by irradiation were sulfur compounds that produced an odor characteristic 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 and others 1999). The degradation of amino acids by oxidative deaminationdecarboxylation via Strecker degradation produces branched chain aldehydes (Mottram and others 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 alkoxyl 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 alkoxyl radical at the C-terminal alphacarbon results in C-terminal decarboxylation (Davies 1996). More than 1 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).

Besides amino acids, fatty acids are also radiolyzed by irradiations. When triglycerides or fatty acids are irradiated, hydrocarbons are formed by cutting CO<sub>2</sub> and CH<sub>3</sub>COOH off from fatty acids in various free-radical reactions. The yield of these radiolytically generated hydrocarbons was linear with absorbed dose (Morehouse and others 1993). Radiolytic degradation of fatty acid methyl ethers were affected by irradiation dose, irradiation temperature, oxygen pressure, and fatty acid components (Miyahara and others 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 and others 2003). Jo and others (1999) showed that 1-heptene content in volatiles was positively correlated to irradiation dose, and Du and others (2001a) showed that the production of alkenes and alkanes, the degradation products of fatty acids, also increased proportionally to irradiation dose.

Irradiation causes some degradation of vitamins. Thiamin (vitamin  $B_1$ ) is the most radiation-sensitive among the water-soluble vitamins. Irradiation induces a slight reduction in thiamine levels in chicken meat, but these incurred losses were unlikely to be of nutritional significance (Graham and others 1998).

#### Irradiation and color

Irradiation can induce a variety of color changes depending

upon irradiation dose, animal species, muscle type, pH, and reducing potential of meat and packaging type. Usually light meat produces pink color whereas dark meat becomes brown or gray after irradiation (Nam and Ahn 2003b). Irradiated turkey and chicken breast meat had a higher *a*\* values, showing an increase in redness (Du and others 2001b; Lewis and others 2002; Nam and Ahn 2002a, 2002b). But for irradiated raw beef, *a*\* values decreased (Nanke and others 1998; Nam and Ahn 2003c), and yellowness increased with dose and storage time (Nanke and others 1998).

The color change induced by irradiation is associated with CO production during irradiation (Nam and Ahn 2003b). Nam and Ahn (2002a, 2002b) showed that irradiation increased the production of CO, which is correlated with the increased redness of irradiated meat. They characterized the pigment that causes pinkness in irradiated turkey meat as carbon monoxide–myoglobin (CO-Mb). The oxidation-reduction potential of meat was lower in electron-beam irradiated meat than that of nonirradiated meat (Du and others 2001b; Nam and Ahn 2002a), which played an important role in the formation of CO-Mb. The production of CO and changes of oxidation-reduction potential in red meats by irradiation were similar to those of light meat, but the different color changes were due to high pigment content in red meat (Nam and Ahn 2003b).

#### Irradiation, water-holding capacity, and texture

Irradiated chicken breasts had more cooking loss than nonirradiated chicken breasts (Yoon 2003). Zhu and others (2004a) found that irradiation significantly increased centrifugation loss of water from pork loins compared with that of nonirradiated samples. The mechanism for irradiation-induced water loss is not clear, but 2 theories exist: (1) irradiation may damage the integrity of membrane structure of muscle fibers (Lakritz and others 1987), and (2) irradiation may denature muscle proteins, thus reducing water-holding capacity (Lynch and others 1991). Transmission electron microscopy showed significant differences 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).

Lewis and others (2002) found that the texture attributes were lower in irradiated (1.0 kGy and 1.8 kGy) chicken breasts 14 d and 28 d after irradiation. Luchsinger and others (1996) reported that irradiation had minimal effects on texture of pork chops. Yoon (2003) found that irradiated chicken breasts had higher shear force than nonirradiated chicken breasts. However, Zhu and others (2005) showed that irradiation had no significant effect on the texture of vacuum-packaged RTE turkey breast rolls 7 d after irradiation.

#### Irradiation and lipid oxidation

Lipid oxidation is the primary cause of quality deterioration in cooked meats. Lipid oxidation forms an oxidation off-flavor characterized as a cardboard, warmed-over, or rancid/painty flavor (Ang and Lyon 1990), induces discoloration and adverse changes in texture, increase drip loss, decreases nutritional value and functionality, and generates compounds that may be detrimental to the health of consumers (Gray and others 1996).

lonizing radiation can initiate oxidation but accelerated lipid oxidation in meat only under aerobic conditions (Du and others 2001a; Ahn and Lee 2004). 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 vacuum-packaging minimizes oxidative changes in meat (Du and others 2001a; Ahn and Lee 2004). Vacuum-packaged irradiated samples, however, retained sulfur volatile compounds responsible for the irradiation off-odor during storage (Ahn and others 2000a; Nam and Ahn 2003a). 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 and others 2001a, 2001b; Nam and others 2001). The amounts of cholesterol oxidation products and lipid oxidation products are closely related to the proportion of polyunsaturated fatty acids in meat (Ahn and others 2001). Polyunsaturated fatty acid-enriched diets increased meat susceptibility to oxidation (Grau and others 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).

#### Antimicrobials and Meat Quality

Papadopoulos and others (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 and others (2003) reported that lactate/diacetate-enhanced chops maintained higher a\* and b\* values during display and had less visual discoloration after 96 h of display. Chops pumped with lactate, acetate, or lactate/diacetate mixture were more tender and juicy and had more pork flavor than controls. Cegielska-Radziejewska and Pikul (2004) showed that SL inhibited the formation of malonaldehyde in sliced poultry sausage during refrigerated storage. Lamkey and others (1991) reported that SL added to fresh pork sausage did not affect lean color but resulted in more rapid surface discoloration. Bradford and others (1993) showed that 2% potassium lactate had no effect on guality and sensory properties of low-fat pork sausage or lean color during refrigerated aerobic storage. Adding 2% SL to turkey breast rolls resulted in lower color a\* and b\* values, but increased hardness, springiness, cohesiveness, chewiness, and resilience of turkey breast rolls (Zhu and others 2004b, 2005). Including 3.3% commercial SL in frankfurter formulation did not affect textural profile of sausage. Addition of potassium sorbate up to 0.1% or sodium benzoate up to 0.1% in products formulation had no effects on the texture of products (Choi and Chin 2003; Zhu and others 2004b, 2005). These results suggested that the effect of SL on the quality of products depends on SL level and product types. A high concentration of SDA has a negative effect on the flavor of ham products (Stekelenburg and Kant-Muermans 2001). However, at lower levels ( $\leq 0.1\%$ ), SDA does not influence the quality of meat products (Stekelenburg 2003; Zhu and others 2004b).

The addition of potassium benzoate greatly increased the content of benzene in the volatiles of irradiated RTE turkey ham and breast rolls, suggesting that benzoate salt is not a good antimicrobial used in products for irradiation (Zhu and others 2004b, 2005).

#### **High-pressure Processing and Meat Quality**

High-pressure processing (HPP) causes minimal changes in "fresh" characteristics of foods because it can be conducted at ambient or refrigerated temperatures. However, there is no doubt that HPP causes quality changes of meat. Some of the changes such as color and lipid oxidation are detrimental, whereas other changes such as pressure tenderization and pressure-assisted gelation are beneficial. An excellent review of HPP on the quality of RTE meats is available (Cheftel and Culioli 1997).

### Summary

L. monocytogenes is a major safety concern for RTE meat products, which are frequently contaminated with this pathogen. Irradiation is an effective post-packaging intervention technology to eliminate L. monocytogenes contaminated RTE meat products; however, it also causes quality problem such as off-flavor, color change, and lipid oxidation, and thus only low dosage irradiation is recommended. Formulating meat products with antimicrobial additives are another approach to suppress the growth of contaminated L. monocytogenes during storage, which results in relatively minor quality problem. However, antimicrobials cannot destroy the pathogen that existed in RTE meat. Combining several intervention technologies such as low-dose irradiation and antimicrobials is a promising technology to ensure the safety of RTE meat products against *L. monocytogenes* without sacrificing the quality of RTE meats.

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