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Development of a novel device for applying uniform doses of electron beam irradiation on carcasses

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ABSTRACT

The Maxim's Electron Scatter Chamber (Maxim Chamber) was developed to obtain uniform dose distribution when applying electron beam (e-beam) irradiation to materials of irregular surface. This was achieved by placing a stainless steel mesh surrounding a cylindrical area where the target sample was placed. Upon contact with the mesh, electrons scatter and are directed onto the target from multiple angles, eliminating the e-beam linearity and resulting in a uniform dose distribution over the target surface. The effect of irradiation in the Maxim Chamber on dose distribution and pathogen reduction was tested on rabbit carcasses to simulate other larger carcasses. The dose uniformity ratio (DUR) on the rabbit carcasses was 1.8, indicating an acceptable dose distribution. On inoculated carcasses, this treatment reduced *Escherichia coli* O157:H7 by >5 log cycles. These results indicate that carcass irradiation using e-beam is feasible using the Maxim's electron scattering chamber. Appropriate adjustments will be further needed for commercial application on beef and other animal carcasses.

1. Introduction

Between 2004 and 2012 thousands of pounds of ground beef have been recalled due to contamination by Shiga toxin-producing *Escherichia coli* O157:H7 (USDA FSIS, 2004-2012). Sources of *E. coli* O157:H7 in beef include the hides of animals entering beef harvest establishments or the intestinal contents, which may cross-contaminate carcasses during the harvest process (Huffman, 2002). The purpose of these recalls is to remove meat from commerce when it is believed to be injurious or harmful for human consumption. In 1994, the USDA's Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 an adulterant in raw ground beef and began sampling processing plants and retail stores (USDA, 1999). In September 2011, FSIS announced that six additional Shiga toxin-producing *E. coli* (STEC) serogroups (O26, O103, O45, O111, O121, and O145) would also be considered adulterants (USDA, 2011a). Symptoms of STEC infections often include stomach cramps, diarrhea (which may develop into hemorrhagic colitis) and vomiting; however, 5–10% of individuals diagnosed with STEC infections may develop hemolytic uremic syndrome (HUS) which may lead to renal failure (5%) and death (3–5%) (Mead & Griffin, 1998). Thrombotic thrombocytopenic purpura, a neurological syndrome, may also occur, effecting mostly elderly adults but is often fatal (Ruggenenti, Noris, & Remuzzi, 2001). The Centers for Disease Control and Prevention (CDC) reported that an average of 2,138 cases and 20 deaths in the U.S. is attributed to this pathogen annually (Scallan et al., 2011). While the incidence of *E. coli* O157:H7 has declined in 2010 to reach the national health objective target of ≤1 case per 100,000 (Gilliss et al., 2011), the estimated societal cost of a single fatal case involving HUS has been estimated at $6.2 million (Frenzen, Drake, & Angulo, 2005).

Beef harvest facilities in the U.S. are required to implement Hazard Analysis and Critical Control point (HACCP) systems for controlling biological, physical and chemical hazards (USDA, 1996). As part of their HACCP plan, many beef harvest facilities have implemented decontaminating treatments including antimicrobial solutions to improve the microbiological safety of their products. A variety of antimicrobial compounds or hot water, and their effects on pathogenic bacteria have been evaluated for use on carcasses (Acuff, 2005; Courantin et al., 2005; Gill, 2009; Nutsch et al., 1998). Some of these antimicrobial solutions have proven to be effective against bacteria on beef carcasses in laboratory settings; however, this success has not always been replicated at commercial processing plants (Edwards & Fung, 2006). Gill (2009) suggested that the discrepancies between laboratory studies and real-life application of antimicrobial solutions to carcasses may be due to factors such as the antimicrobial solutions becoming diluted when applied to wet carcasses or, sufficient amounts of the sanitizing solution not being applied, due possibly to costs, which might result in a non-uniform application. It is well recognized that pathogens allocated in crevices or carcass cuts may not be reached by the antimicrobial solution, resulting in some pathogens remaining viable in carcasses that were subjected to antimicrobial interventions (Acuff,

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2005). Despite the wide use of pathogen interventions using antimicrobial solutions in beef harvest operations, recalls and outbreaks linked to meat obtained from carcasses that were subjected to such pathogen interventions continue to occur. E-beam irradiation has been proposed as an alternative that would resolve the limitations of interventions using hot water or chemical antimicrobials (Ehlermann, 1993). Multiple studies have proven that ionizing irradiation can significantly reduce pathogens including *E. coli* O157:H7, *Salmonella*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Listeria monocytogenes* in meat and poultry products without damaging the quality of these products (Arthur et al., 2005; Caja, Ruiz del Castillo, & Blanch, 2008; Chiasson, Borsa, & Lacroix, 2005; Chiasson, Borsa, Ouattara, & Lacroix, 2004; Clavero, Monk, Beuchat, Doyle, & Brackett, 1994; Duong et al., 2008; Fu, Sebranek, & Murano, 1995; Gilliss et al., 2011; Ismail, Lee, Ko, & Ahn, 2008; Kundu & Holley, 2013; Kwon, Kwon, Nam, Lee, & Ahn, 2008; Lefebvre, Thibault, Charbonneau, & Piette, 1994; Lopez-Gonzalez, Murano, Brennan, & Murano, 1999, 2000; Molins, Motarjemi, & Käferstein, 2001; Murano, 1995; Olson, 1998; Park et al., 2010; Schilling et al., 2009; Thayer & Boyd, 1993).

E-beam irradiation of beef surfaces has been studied and found to be a promising alternative (Arthur et al., 2005). Due to the poor penetration power of e-beams, carcasses would be irradiated only on the surface and at low depths. Likely, this would permit the trimming of the surface structures, such as fat and other external tissues to remove all portions that actually received the e-beams from carcasses. Based on these studies, the American Meat Institute (AMI) submitted a petition for e-beam irradiation to be approved as a processing aid in beef carcasses (AMI, 2005). This petition was later denied by FSIS based on concerns about the inability of e-beams to be applied evenly over surfaces of irregular shape; therefore the industry not being able to ensure an even dose distribution if carcasses were irradiated, or to ensure that the total absorbed dose did not exceed the maximum approved absorbed dose (USDA, 2011b).

To enable e-beam irradiation of foods with irregular shapes such as carcasses, while achieving an even dose distribution over the entire surface, a novel device, the Maxim's Electron Scatter Chamber (Maxim Chamber) was designed to take advantage of the natural electron scattering that occurs when electrons collide with any surface. The materials in the Maxim Chamber are designed to promote electron scattering, which will make the electrons to travel in a randomly linear fashion to any point of the surface placed in the center of the cylinder. As a result, the target surface will receive an even amount of electrons despite its shape (Maxim, Neal, & Castillo, 2011). The objectives of this study were to test the dose distribution on the surface and subsurface of rabbit carcasses simulating larger animal carcasses, after e-beam treatment in the Maxim chamber, and to determine the effect of this treatment in the reduction of *E. coli* O157:H7 inoculated onto rabbit carcasses.

2. Material and methods

2.1. Maxim chamber

The Maxim Chamber consists of a cylindrical metallic structure that has a galvanized wire grid jacket inside followed by a core unit to place the samples to be treated. The jacket is filled with a stainless steel mesh providing a virtually infinite number of angles for electrons to scatter in every direction. The core is covered on both sides by attenuators, which eliminate the dose of energy into the chamber from the direct electron beam. The electrons hitting the stainless steel mesh will be scattered in a virtually infinite number of angles. As a result, some of these scatter electrons are applied onto the material located in the core of the chamber from all directions, eliminating the linearity of the electron beam. Stainless steel rods were placed inside the chamber to hold the materials irradiated in place (Fig. 1).

2.2. Carcass preparation

Due to the size of the electron beam scanning horn, beef carcasses were too large to fit in the E-beam irradiation facility at Texas A&M University. Therefore rabbit carcasses were chosen to simulate the shape of beef carcasses. Fresh rabbit carcasses typical of this type of meat entering the U.S. food supply were obtained from a meat distributor in Houston, TX. After purchasing, rabbit carcasses were placed in a cooler with an internal temperature of 4 °C for 2 h and transported 100 miles directly to the Texas A&M Food Microbiology Laboratory, where they were stored at 4 °C for up to 24 h.

The study was conducted in two trials, each including 3 carcasses ($N = 6$). In addition, dosimetry studies were conducted on another set of 3 carcasses to determine dose distribution and dose uniformity ratio (DUR). In addition, one carcass was kept frozen and used for repeated trials in determining the percent dose recovery as described below.

2.3. Bacterial cultures

Rifampicin-resistant (Rif^+) variants were derived from 5 parent strains of *E. coli* O157:H7 obtained from the Texas A&M Food Microbiology Laboratory (College Station, TX) culture collection, following the method described by Kaspar and Tamplin (1993). Growth curves and irradiation sensitivity of the Rif^+ strains were determined to be virtually indistinguishable from the parent strains. Five strains of Rif^+ *E. coli* O157:H7 were cultured onto tryptic soy agar slants (TSA; Difco, Becton Dickinson, Sparks, MD) and incubated at 37 °C for 24 h. Three days prior to each experiment the microorganisms were resuscitated by two consecutive transfers to tryptic soy broth (TSB; Difco) and incubated at 37 °C for 12 h. Rifampicin resistance was confirmed by streaking TSB cultures onto plates of TSA + 100 mg/liter rifampicin (Sigma, St. Louis, MO; rif-TSA) and incubated at 37 °C for 24 h.

2.4. Inoculum preparation and inoculation

Nine milliliters of a 12 h culture of each microorganism was dispensed in sterile centrifuge tubes (15 ml) and harvested by centrifugation at 1623 ×*g* in a Jouan B4i centrifuge (Thermo Electron Corp., Madison, WI) for 15 min at 21 °C. The pellet for each microorganism was resuspended in 5 ml of 0.1% peptone water (Difco) and 1 ml aliquots of each were combined to make a bacterial suspension in a sterile bottle containing 95 ml 0.1% peptone water. The mean bacterial concentration in this inoculum was determined to be 7.9 log CFU/ml. The inoculum was kept at room temperature (23–24 °C) and was used within 2 h after preparation. Six rabbit carcass regions (hindquarter top exterior, neck (trial 1) or forequarter side exterior (trial 2), forequarter top exterior, hindquarter belly interior, forequarter belly interior and hindquarter top) were inoculated for each carcass by outlining 2 10-cm^2 areas using colored pins and then adding 100 μl of the inoculum onto each 10-cm^2 area and spread with a sterile bent glass rod. The target level of *E. coli* O157:H7 inoculated on the carcass was 6 log CFU/cm^2. On trial 1, sub-superficial inoculation was achieved by cutting a slit on the neck to a depth of approximately 2 mm, placing a piece of cotton gauze embedded with 6 log CFU of *E. coli* O157:H7 inside the cut and then replacing the tissue to maintain the inoculated gauze covered by the meat tissue to simulate pathogen allocation under the carcass surface in cuts or crevices. Inoculated cotton gauze was used to determine if the e-beam irradiation was penetrating the surface of the carcass in a manner in which a known amount of inoculum pre and post treatment could be enumerated. After treatment, the cotton gauze piece was extracted and used as sample for bacterial counts.