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Application of X-ray for inactivation of foodborne pathogens in ready-to-eat sliced ham and mechanism of the bactericidal action

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ABSTRACT

The aim of this study was to investigate the effect of X-ray irradiation in reducing the population of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in ready-to-eat (RTE) sliced ham and to verify the mechanisms underlying the lethal effect of X-ray irradiation. In addition, the effect of the X-ray treatment on food quality was determined by measuring color and texture changes. Sample surfaces were inoculated with cocktails of three pathogens and subjected to X-ray irradiation, with doses ranging from 0.2 to 0.8 kGy. After 0.8 kGy of X-ray irradiation, the numbers of S. *Typhimurium*, *E. coli* O157:H7, and *L. monocytogenes* were reduced by 5.7, 7.2, and 6.9 log CFU/g, respectively, without generating sublethally injured cells with potential to recover. The mechanism of X-ray-associated lethality was determined using fluorescent staining. We confirmed that the primary factors contributing to the lethal effect of X-ray treatment are related to intracellular enzyme inactivation and damage to cellular DNA rather than disruption of the cell membrane. Following X-ray irradiation, color values and textural characteristics of sliced ham products were not significantly altered compared to that of the control. The results of this study suggest that X-ray irradiation can be potentially used as a novel non-thermal process for inactivating foodborne pathogens in post-packaged RTE deli food products without compromising product quality.

1. Introduction

Deli meat is one of the most consumed foods worldwide; in particular, precooked sliced ham is appreciated because it is easy to use and convenient to store (Fernando, 2017; USDA, 2017). However, convenience food such as sliced ham forms a new contamination route for food pathogens (Rajkovic, Tomasevic, Meulenaer, & Devlieghere, 2017). Owing to processes such as cutting, slicing, and packing, sliced ham can be easily contaminated by various foodborne pathogens (Cabeza et al., 2009). Sheen and Hwang (2010) confirmed that *Escherichia coli* O157:H7 could be transferred from surfaces of meat cutters to the ready-to-eat (RTE) meat product surfaces. In 2002, eight people died because of a multistate listeriosis outbreak that was linked to deli meats in the USA (CDC, 2002). Between April and June 2010, an outbreak of *Salmonella* Typhimurium was identified in Denmark, which was traced back to deli meat products. The outbreak involved twenty cases, 50% of which were children aged 10 years or younger (Kuhn, Torpdahl, Frank, Sigsgaard, & Ethelberg, 2011).

To control pathogenic microorganisms on sliced ham, several non-thermal processes such as use of pulsed ultraviolet light (Wambura et al., 2011), high pressure (Clariana et al., 2011), and sodium nitrite treatment (Tompkin, 2005) have been evaluated. However, pulsed ultraviolet light treatment easily deteriorated the quality of sliced ham, particularly color values (Wambura et al., 2011). Structural modifications that changed fat and moisture content, color, texture, and flavor of dry-cured ham occurred with high pressure treatment (Clariana et al., 2011). Sodium nitrite has been mainly used for fixing color and inhibiting microbial growth in meat products. However, sodium nitrite binds to amines in meat at high temperature and produces nitrosamine, a carcinogen. In addition, nitrite is ineffective in inactivating Gram-negative pathogens. Since a nitrate reductase is required to reduce nitrate to nitrite, Gram-negative pathogens can assimilate inorganic nitrogen from nitrates and nitrites under anaerobic conditions (Tompkin, 2005). Consequently, a new sterilization treatment is required to efficiently control major pathogenic microorganisms including *S.* Typhimurium and *E. coli* O157:H7 in sliced ham products.

Ionizing radiation is also an effective method of inactivating pathogens in food products (Mahmoud, 2009a). A joint Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA)/World Health Organization (WHO) Expert Committee reported

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that no toxicological, microbiological, and nutritional problem occurred with the use of up to 10 kGy irradiation in food (WHO, 1981). The Food and Drug Administration (FDA) allows the use of ionizing radiation to a dose up to 4.5 kGy for controlling foodborne pathogens and extending the self-life of refrigerated meat products (FDA, 2017). Cabeza, Cambero, Hoz, and Ordo´n˜ez (2007) reported that electron beam (E-beam) irradiation at a dose of 1 kGy meets the food safety objective required by the EU and USDA (10^2 CFU for *L. monocytogenes*) in vacuum packed RTE cooked hams without noticeable changes in the sensory quality. Park et al. (2010) showed that gamma ray irradiation at 5–10 kGy on beef sausage patties could be useful in reducing bacterial populations with no adverse effect on quality and most of sensory characteristics. Among ionization radiations, X-ray, a form of electromagnetic radiation, is generated when the high velocity electrons collide with a metallic anode. Owing to its penetrability, X-rays are generally used to image the interiors of objects in medical radiography (Whaites, 2013, pp. 15–20). X-rays and gamma rays are similar with respect to penetration power but are classified according to their origin. Gamma rays arise from the nucleus of an atom, whereas X-rays originated from extranuclear electrons (L'Annunziata, 2012). Therefore, shielding from gamma rays is essential to prevent the radioactive decay of atomic nuclei, which entails enormous costs for nuclear waste disposal. In addition, the general attitude of consumers towards gamma ray-irradiated food products is considerably negative (Sádecká, 2007). For these reasons, many researchers have recently started applying X-rays as an alternative to gamma rays for food treatment.

Mahmoud (2009b) reported that the population of foodborne pathogens decreased to less than the detectable limit when ready-to-eat shrimp inoculated with *E. coli* O157:H7, *Salmonella enterica*, *Shigella flexneri*, and *Vibrio parahaemolyticus* was treated with X-rays. In addition, the effect of low-energy (70 kV) X-ray radiation for inactivating *E. coli* O157:H7 on lettuces was investigated by Jeong, Marks, Ryser, and Moosekian (2010), who concluded that the D_{10}-value of 0.04 kGy X-ray irradiation is 3.4 times lower than that of 0.136 kGy gamma irradiation. However, currently, studies on the use of X-ray radiation for inactivating pathogenic microorganisms in deli meats is lacking.

The objectives of this study were to investigate the bactericidal effect of X-rays for inactivating *S*. Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* in sliced ham and to assess the quality changes of the product after X-ray irradiation. Despite *E. coli* O157:H7 not being a common concern in deli meat, it was used to demonstrate X-ray effects. In addition, ionizing radiation causes water molecules to lose an electron, producing hydroxyl radicals and hydrogen peroxide. Hydroxyl radicals and hydrogen peroxide are known to damage DNA (Simic, 1982; Ward, 1988). However, the specific mechanisms via which X-ray irradiation exert bactericidal effects has not yet been identified. Therefore, another purpose of the present study was to examine the mechanism underlying the lethal effect of X-ray irradiation.

2. Materials and methods

2.1. Bacterial strains

Three strains each of *S*. Typhimurium (ATCC 19585, ATCC 43971, and DT 104), *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), and *L. monocytogenes* (ATCC 15313, ATCC 19111, and ATCC 19115), obtained from the bacterial culture collection of Hankyong National University (Anseong, South Korea), were used. Stock cultures were frozen at −80 °C by mixing 0.7 ml of tryptic soy broth (TSB; MB Cell, CA, USA) with 0.3 ml of 50% glycerol. Working cultures were streaked onto tryptic soy agar (TSA; MB Cell), incubated at 37 °C for 24 h, and stored at 4 °C.

2.2. Preparation of pathogens

Each strain of *S*. Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* were cultured in 5 ml TSB with 0.6% yeast extract (YE; Difco, Becton Dickinson, Sparks, MD, USA) at 37 °C for 24 h, followed by centrifugation (3200 rpm for 20 min at 4 °C), and the supernatant was discarded. The final cell pellets were resuspended in 9 ml 0.2% sterile peptone water (PW; Difco) to obtain approximately 10^7–10^8 CFU/ml. Mixed culture cocktails were prepared by blending together equal volumes of each test strain. These cocktails were used in an inactivation study at a final concentration of ca. 10^7 CFU/ml. To analyze the mechanism of inactivation, each final pellet of *S*. Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* was resuspended in 15 ml phosphate-buffered saline (PBS; 0.1 M).

2.3. Sample preparation and inoculation

Precooked and vacuum-packaged sliced ham (approximately 90 by 90 by 2 mm) was purchased from a local grocery store (Anseong, South Korea) and maintained in a refrigerator (4 °C) within 2 days. Next, each sliced ham was placed on a sterile aluminum foil and spot inoculated with 100 μl of the mixed pathogen suspension on one side of the sliced ham. Then, the inoculum was spread with a sterile plastic spreader for even distribution of pathogens, and the samples were dried inside a biosafety hood for 10 min without the fan running to avoid excessive surface aridity. The inoculated samples were repackaged with their own polyethylene (PE) films.

2.4. X-ray treatment and dosimetry

The prepared samples were irradiated in an X-ray irradiator (CP-160 Cabinet X-Radiato System, Faxitron Inc., AZ, USA) at the Korea Atomic Energy Research Institute (Jeongeup, South Korea). The X-ray tube (MXR-160, Comet, Switzerland), which consisted of stationary anode, tungsten target, and metal ceramic with beryllium window (0.8 mm thick) was used as an X-ray emitting source. The doses applied in this study were 0 (control), 0.2, 0.4, 0.6, and 0.8 kGy and the applied doses were adjusted by treatment time. For X-ray irradiation, samples per dose were irradiated perpendicularly to the incident radiation and were placed in the center of the treatment chamber. The vertical distance between the emitter and samples was approximately 99 cm. Non-irradiated controls were also maintained under the same storage and transport conditions as the irradiated samples. The applied doses were adjusted by treatment time. Dosimetry was performed using alanine pellet dosimeters (Bruker Biospin, Rheinstetten, Germany) and an e-scan alanine dosimeter reader (Bruker Biospin). A dosimeter was packaged in a polyethylene bag to prevent moisture absorption and four dosimeters were attached to the top of samples for measuring each irradiation dose. To determine the mechanism of inactivation, 3 ml of each cell suspension was transferred to a sterile 12-well flat-bottom polystyrene plate (SPL, South Korea) and irradiated with a dose of 0.4 and 0.8 kGy under identical conditions. The volume of the cell suspension (3 ml) and the treatment doses (0.4 and 0.8 kGy) were selected by performing preliminary experiments. The actual doses were verified by attaching a dosimeter on the top of a 12-well plate.

2.5. Bacterial enumeration

Each treated sliced ham (approximately 25 g) was transferred into sterile stomacher bags (Labplas Inc., Quebec, Canada) containing 100 ml PW and homogenized for 2 min with a stomacher (HBM-400B, HBM Biomed, Tianjin, China). After homogenization, aliquots (1 ml) of stomached samples were 10-fold serially diluted in 9 ml 0.2% PW, and 100 μl of the diluent was spread onto each selective medium. Sorbitol MacConkey agar (SMAC; MB Cell), xylose lysine desoxycholate agar (XLD; MB Cell), and Oxford agar base (OAB, MB Cell) with Bacto Oxford antimicrobial supplement (MB Cell) were used as selective media for the enumeration of *S*. Typhimurium, *E. coli* O157:H7, and *L. monocytogenes,* respectively. All agar media were incubated at 37 °C for

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