Effects of Irradiation on Survival and Growth of *Listeria Monocytogenes* and Natural Microflora in Vacuum-packaged Turkey Hams and Breast Rolls

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Meijun J. Zhu, graduate research assistant; Aubrey Mendonca, professor of food science and human nutrition; Hesham A. Ismail, graduate research assistant; Dong U. Ahn, professor of animal science

Summary and Implications

The D_{10}-values of *L. monocytogenes* in breast rolls and hams were 0.52 and 0.47 kGy, respectively. For breast rolls, the $\log_{10}$ reductions of *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 $\log_{10}$ reductions of APC in breast rolls following 1.0 and 2.0-kGy irradiation were 2.9 and 5.2 while that of hams was < 10 CFU/cm^2 after 1.0- and 2.0-kGy irradiation. In 2.0 kGy-irradiated hams, *L. Monocytogenes* grew to 4.82 $\log_{10}$ CFU/cm^2 after 28 d storage at 4 °C, while APC increased to 2.98 $\log_{10}$ CFU/cm^2, respectively. In breast rolls after 14 d storage, APC in 1.0 kGy-irradiated samples increased to 7.53 $\log_{10}$ CFU/cm^2; and APC increased to 2.63 and 4.68 $\log_{10}$ CFU/cm^2 for 2.0 kGy-irradiated breast rolls after 14 and 28 d storage. However, during the storage of breast rolls, *L. monocytogenes* grew slowly or even stopped to grow in both non-irradiated and irradiated breast rolls due to the competitive inhibition of natural flora in breast rolls.

Irradiation greatly reduced *L. monocytogenes* and APC in turkey hams and breast rolls. However, at least 2.5 kGy irradiation is needed to achieve a 5-log reduction of *L. monocytogenes* in turkey hams and breast rolls. Some cells survived irradiation and grew during storage after lag phase. To control *L. monocytogenes* contamination in RTE turkey hams and breast rolls during storage, additional barriers, such as adding preservatives, are necessary in order to ensure the microbial safety of products following low-dose irradiation.

Introduction

L. 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. *L. monocytogenes* is commonly found in natural environment, the intestinal tract of infected animals, food processing environments and catering. In the United States, *L. monocytogenes* was found in 5.9% of turkey carcass rinses and in 31% of ground turkey meat. 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. 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. 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. In 2000, a multistate outbreak involving deli turkey meat results in 29 cases, 4 death and 3 miscarriages or still birth. 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. 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. 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 supplemented with 0.6% yeast extract (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. 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

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 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. The samples were surface inoculated with 0.1 ml *L. monocytogenes* cocktail stock suspension to a level of 10^6 CFU/cm^2, and then vacuum-packaged in nylon-polyethylene bags. The packaged samples were further separated randomly into five groups and irradiated at 0, 1.0, 1.5, 2.0, or 2.5 kGy. 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 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} CFU/cm^2) in inoculated sample at each irradiation level was plotted against irradiation dose to construct the survivor curves of *L. monocytogenes.* The D_{10} value 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. 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. Numbers of naturally-occurring bacteria on sliced turkey hams were 5.23×10^2 CFU/cm^2. During 28 d 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/cm^2. After 28 refrigerated storage, APC in 2.0 kGy-treated hams increased to 2.98 $\log_{10}$ CFU/ cm^2, equivalent to the starting APC level. For 1.0 kGy-irradiated samples, the equivalent starting APC level was achieved at 14d. The numbers of natural flora on sliced turkey breast rolls were about 6.22 $\log_{10}$ CFU/cm^2, which is quite high. After storage at 4 °C for 7 d, APC increased to 7.79 $\log_{10}$ CFU/cm^2, and remained at this high level during 28 d storage. Irradiation at 1.0 and 2.0 kGy reduced natural microflora to 3.33 and 0.98 $\log_{10}$ CFU/cm^2, respectively. After irradiation, APC in 1.0 kGy-treated breast rolls increased rapidly, which increased to 7.53 $\log_{10}$ CFU/cm^2 after 14 d storage. The APC in 2.0 kGy-treated breast rolls increased to 4.68 after 28 d storage.

Irradiation at 1.0 and 2.0 kGy reduced *L. monocytogenes* by 1.89 and 3.91 $\log_{10}$ CFU/cm^2, respectively. *L. monocytogenes* numbers in non-irradiated turkey hams increased about 1 log during the first 7 d storage, then the organisms remained at peak population of 7-8 $\log_{10}$ CFU/cm^2 during 28 d storage 4°C. No increase in numbers of survivors occurred in 1.0 kGy-irradiated turkey hams until after 7 d. In 2.0 kGy-irradiated turkey hams, the growth of *L. monocytogenes* was retarded for about 2 weeks. After 28 d refrigerated storage, *L. monocytogenes* survivors in 1.0 kGy- and 2.0 kGy-treated turkey hams increased to 6.41 and 4.82 $\log_{10}$ CFU/cm^2, respectively.

One- and 2.0 kGy-irradiation reduced *L. monocytogenes* by 1.47 and 3.52 $\log_{10}$ CFU/cm^2, respectively. No growth of *L. monocytogenes* was observed in both non-irradiation and 1.0 kGy-treated breast rolls during the whole 28 d storage at 4°C. In 2.0 kGy-irradiated breast rolls, the number of *L. monocytogenes* survivors reduced by 1.35 $\log_{10}$ CFU/cm^2 during the first 14 d storage, then *L. monocytogenes* grew slowly and the survivors increased to 2.71 $\log_{10}$ CFU/cm^2 by the end of 28 d storage. 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. 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*.

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-d 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.