Impact of Antimicrobial Ingredients and Irradiation on the Survival of *Listeria monocytogenes* and the Quality of Ready-to-Eat Turkey Ham

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Summary and Implications

Antimicrobial ingredients [2% sodium lactate (SL), 0.1% sodium diacetate (SDA), 0.1% potassium benzoate (PB)] and low-dose irradiation were combined and tested for their effects on the growth of *L. monocytogenes* and 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 (PSS). Addition of SL+SDA or PB+SL in combination with 1.0 kGy irradiation was effective in suppressing the growth of *L. monocytogenes* for about six weeks when stored at 4 °C, while 2.0-kGy-irradiation was listeriostatic. One kGy irradiated ham with PSS was listeriostatic throughout storage. SL increased firmness of turkey hams and sensory panelists noted that the saltiness was a little higher in products containing SL, but its overall impact on quality was minimal. Amounts of benzene were detected in irradiated hams with PB, showing PB was not fit as an antimicrobial ingredient 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*.

Introduction

Ready-to-eat (RTE) meat products are occasionally contaminated with *Listeria monocytogenes* mostly due to post-processing contamination. Due to its ability to grow at refrigerated temperature and its resistance to salt and nitrite, any *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%) and economic impact due to products recall, *L. monocytogenes* is still a major food safety issue for processed meat industry. Currently, the U.S. Department of Agriculture establishes a "zero tolerance" policy for *L. monocytogenes* in RTE meat products. The ubiquitous nature of *L. monocytogenes* and its ability to grow at refrigerated temperature makes thermal processing and refrigerated storage insufficient to provide safety margin for processed meat products. To ensure microbiological safety, therefore, additional hurdles are needed.

Irradiation, one of several post-package decontamination technologies, is an effective way of destroying vegetative foodborne pathogens, including *L. monocytogenes*. Although effective in controlling microorganisms, irradiation negatively affects the quality of RTE meat even at 2.0 kGy. Because of this, only low-dosage irradiation would be practiced by meat industry in order to minimize quality changes. With low-dose irradiation, however, some pathogens can survive and proliferate in RTE meats during storage. Thus, it is necessary to use antimicrobial ingredients 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, but there is limited information on the effectiveness of antimicrobial ingredients combined with irradiation in inhibiting the growth of *L. moncytogenes* in RTE meat products.

The objectives of this study were to determine the effect of irradiation in combination with antimicrobial ingredients on the survival and growth of *L. monocytogenes* in RTE turkey hams during refrigerated storage. The effects of antimicrobial additives and irradiation on the organoleptic quality of RTE turkey hams were also examined to assess the feasibility of using antimicrobial ingredients and irradiation combinations 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 a 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 incubated at 35 °C for another 18 h. Each strain was harvested by centrifugation, washed twice, and re-suspended in sterile 0.1% (W/V) peptone water. Inoculation cocktail was prepared by mixing equal volumes of each of the five strain suspensions, which has approximately the same number of bacterial population.

Preparation of RTE turkey meat products

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 (PSS) were mixed with meat and other ingredients, and then stuffed into large fibrous casings (ϕ = 11.5cm). The turkey hams were heat processed and then sliced with an electric slicing machine. Sliced samples were vacuum-packaged individually. For volatile analysis and sensory evaluation, only four antimicrobial additive treatments (control, PB+SL, SL+SDA or PSS) were used.

Inoculation of test samples

The sliced turkey hams (2-mm-thick) were aseptically removed from the original bulk package into nylon-polyethylene bags (O_2 < 0.6cm^3/100 in^2/24 h at 38 °C), one slice per bag. One side of each sliced turkey ham was surface inoculated with 0.1 ml *L. monocytogenes* cocktail to a level approximately 10^6 CFU/cm^2. Inoculated turkey ham samples were manually rubbed for 30 s to distribute the inoculum evenly, then vacuum sealed by Multivac, and kept refrigerated overnight prior to irradiation.

Irradiation

All samples were irradiated at refrigerated temperature (4 °C) using a Linear Accelerator Facility. The vacuum-packaged inoculated samples of each additive treatment were divided randomly into five groups, irradiated at 0, 1.0, 1.5, 2.0, or 2.5 kGy, and stored for up to 42 days at 4 C. The number of *L. monocytogenes* survivors in samples 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 cm^2) followed by pummeling in a stomacher for 1 min at medium speed. Samples were serially diluted with 0.1% peptone water and surface-plated (0.1 ml) in duplicate on modified oxford (MOX) agar plates[1] to enumerate *L. monocytogenes*. Typical *Listeria* colonies on MOX plates were counted after 48 h incubation at 35°C.

Sensory evaluation

Turkey hams used for sensory evaluation were manufactured, sliced and irradiated separately. After irradiation, the sliced vacuum packaged RTE turkey hams free from pathogen area was reported as the amount of volatiles released.

Texture profile analysis

The RTE turkey hams were immobilized between specially constructed stainless steel plates, a star-shaped, cherry-pitter probe was used to penetrate the slices perpendicularly. Each sample under went 2 cycles of 50% compression using the above probe fitted to a TA-XT2i® Texture Analyzer. 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.

Statistical analysis

A factorial design was used in this study. Data were analyzed by the General Linear Model of Statistical Analysis System. The differences in the mean values were compared by the Tukey's multiple comparison, and mean values and standard deviation were reported ($P<0.05$).

Results and Discussion

Irradiation sensitivity of Listeria monocytogenes Log_{10} reductions of *L. monocytogenes* in turkey hams following 1.0 to 2.5 kGy irradiation ranged from 2.0 to 5.0. The calculated D_{10}-value for control, PB-, SL- and PB+SL-added turkey ham was about 0.52 kGy, while those for the turkey hams containing SL+SDA and PSS were approximately 0.49 and 0.48, respectively, which is consistent with previous reports. 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.

Effect of antimicrobial ingredients on the growth of L. monocytogenes survived irradiation

Single or combined antimicrobial ingredients delayed the growth of *L. monocytogenes* in turkey ham products. In control turkey ham, *L. monocytogenes* reached the peak number after 21 days of refrigerated storage, while in turkey hams containing two or three combined antimicrobial ingredients the number increased less than one log after 42 days. Although the growth of *L. monocytogenes* in turkey hams with SL+SDA was slower than that of PB+SL, the difference was not significant. The combination of PB+SL+SDA was more effective in controlling the growth of *L. monocytogenes* than PB+SL. The synergistic inhibitory effect of lactate and diacetate

combination on the growth of pathogenic organism has been well documented.

An extended lag phase was observed in turkey hams irradiated at 1.0 and 2.0 kGy and stored at 4 °C, especially for those hams with combined antimicrobial ingredients. The increased lag phase was associated with irradiation dose, and addition of antimicrobial ingredients in turkey formulation greatly increased lag phase at each irradiation dose. During lag phase, surviving pathogens are believed to repair injuries caused by irradiation, and the organisms presumably needed more time to repair irradiation damages when two or three antimicrobial ingredients were present.

For control hams without antimicrobial ingredients, 1.0 kGy and 2.0 kGy delayed the lag phase for 7 and 14 days, respectively. For turkey hams formulated with two antimicrobial ingredients such as 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, and *L. monocytogenes* stayed in lag phase throughout the storage in 2.0-kGy-irradiated hams. In hams with three combined antimicrobial ingredients, 0.1% PB plus 2% SL plus 0.1% SDA, *L. monocytogenes* stayed in the lag phase during the whole refrigerated storage in either 1.0 or 2.0 kGy irradiated hams.

After the lag phase, the surviving pathogens started to proliferate in 1.0 kGy-irradiated control hams and reached a peak of 7.2 $\log_{10}$ CFU/cm^2 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. However, 1.0 kGy irradiation in combination with 2% SL+0.1% SDA or 0.1% PB+2% SL inhibited the growth of *L. monocytogenes* for about six weeks at 4 °C, and 2.0 kGy irradiation in combination with 2% SL+0.1% SDA or 0.1% PB+2% SL was listeriostatic. For hams receiving 1.0 or 2.0 kGy irradiation, adding 0.1% PB+2% SL+0.1% SDA was listeriostatic throughout storage.

Effect of antimicrobial ingredient and irradiation on the quality of turkey hams

A total 43 volatiles were identified by GC-MS. Irradiation increased the amounts of hexane, 3-methyl butanal, 1-heptene, dimethyl disulfide and total volatile contents in turkey ham. Addition of antimicrobial ingredients had no effect on the volatiles of hams with or without irradiation, except for hams containing PB. Irradiation greatly increased the amount of benzene detected in volatiles in hams containing PB. Since benzene has negative effect on health, PB+SL and PSS may not be good antimicrobial combinations for food that received irradiation despite their effectiveness in inhibiting the growth of *L. monocytogenes.*

Microbial experiment showed that 1.0 kGy irradiation plus combined antimicrobial ingredients was effective in inhibiting the growth of *L. monocytogenes*. Therefore**,** 2.0-kGy irradiation was 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 antimicrobial ingredients was significantly higher than that of control. This result indicated that salt level in formulation should be reduced slightly for hams formulated with antimicrobial ingredients.

For all hams containing SL, the hardness is largely higher than the rest of hams. The chewiness of products containing SL was also increased. This should be due to the improved protein gelation during cooking, because 2% SL addition increased the ion content and improved protein solubility and water-binding capacity of the meat. If the increase in hardness and chewiness is undesirable in some products, slightly reduction of phosphate or binders, such as transglutaminase, in formulation should solve the problems.

In summary, irradiation is very effective in reducing *L. monocytogenes* in RTE turkey hams, but survived *L. monocytogenes* could proliferate during subsequent storage. Antimicrobial ingredients combinations (PB+SL, SL+SDA and PSS) could be an additional hurdle to low-dose irradiation of RTE meat to inhibit the growth of surviving pathogens during prolonged refrigerated storage. Sensory properties of turkey hams were not significantly affected by antimicrobial additives combined with 1.0 kGy irradiation. Because a significant amount of benzene was detected in volatiles of hams containing PB, addition of PB as an antimicrobial agent in irradiated foods is not recommended. SL+SDA in combination with 1.0- or 2.0-kGy irradiation, thus, is recommended to ensure safety and quality of RTE ham.