EFFECT OF ELECTRON BEAM IRRADIATION ON MICROBIAL SAFETY OF BEEF INTESTINE IN DIFFERENT PACKAGEING CONDITIONS

Haelim Lee[1], Hyun Joo Kim[1], Hae In Yong[1], Dinesh D. Jayasena[2], Hyun Jung Lee[1], Ki Chang Nam[3], Kyung Haeng Lee[4], Cheorun Jo[1*]

[1]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

[2]Department of Animal Science, Uva Wellassa University, Badulla 90000, Sri Lanka

[3]Department of Animal Science and Technology, Sunchon National University, Suncheon, 540-742, Korea

[4]Department of Food and Nutrition, Korea National University of Transportation Jeungpyung 368-701, Korea

Abstract – The effect of the irradiation against foodborne pathogens *(Escherichia coli* O157:H7 and *Listeria monocytogenes*) on beef edible intestines was investigated. Small intestine and large intestine of beef were packaged in aerobic and vacuum conditions and electron beam irradiated. Irradiation significantly reduced the numbers of the tested pathogens on beef intestines. No viable cells were detected in vacuum- and aerobic- packaged samples at doses of 4 and 3 kGy, respectively. The intestines packed under vacuum had higher D_{10}-value but no significant difference was observed between D_{10}-values of *E coli* O157:H7 and *L. monocytogenes*. Results suggest that low dose of electron beam irradiation can significantly improve the microbial quality of beef intestines during processing.

Key Words – Electron beam, Pathogen, Beef intestine, D_{10}-value

I. INTRODUCTION

Meat byproducts have characteristic sensory traits compared to meat from muscle. Therefore, not only consumption of meat but also edible meat byproducts has been increased [1]. *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Clostridium perfringens*, and *Campylobacter* spp. could be present as notable hazardous pathogens to humans and cause public health concerns. These kinds of pathogens could be detected in meat byproducts. They may originate from the environment of the slaughter house, the digestive track of the slaughtered animals, or unhygienic processing conditions even if chlorine, organic acids, or trisodium phosphate are used to control the growth of microorganisms [2, 3, 4].

Irradiation can be used to increase the food safety by reducing microbial growth and extending the shelf life of foods. The commonly used irradiation sources are gamma ray and electron beam. Gamma ray produced by radionuclides (^{60}Co or ^{137}Cs) have a high penetrating power, while a machine source produce electron-beams (EB) and it has low but effective penetration. EB has advantages that it has lesser influence on the food quality and it is consumer friendly due to non-use of radioisotopes in food industry [5]. Several studies explained that electron-beam irradiation significantly reduces the microbial counts in raw meats and meat products [6, 7, 8]. Although the effect of EB on meat and meat product is well-known, the information on irradiated meat byproducts is still insufficient. The knowledge of bactericidal effect of irradiation will be helpful to implement the EB technology in improving microbial safety of meat byproducts.

Thus, the main objective of this study was to determine inactivation effect of EB irradiation against *E. coli* O157:H7 and *L. monocytogenes* inoculated in beef small and large intestine.

II. MATERIALS AND METHODS

Sample preparation and sterilization

Beef small and large intestine were purchased from commercial market in Daejeon, Korea. Each byproduct was vacuum-packaged and sterilized by using EB irradiation (35 kGy at 10 MeV) with a linear EB RF accelerator (EB Tech, Daejeon, Korea) before the test.

Test pathogens and culture condition

E. coli O157:H7 (KCCM 40406) and *L. monocytogenes* (KCTC 3569) were cultivated and inoculated on the beef intestine (5 g). Half of samples were vacuum-packaged (VP) in low-density polyethylene/nylon vacuum bags (20 × 20 cm) and half were aerobically-packaged (AP) in polyethylene bags (20 × 20 cm).

EB irradiation

Each prepared sample was irradiated on both sides in a linear EB RF accelerator (10 MeV, EB Tech.) and conditions for irradiation are shown in Table 1. The calculated maximum and minimum dose ratio was less than 1.004 for all samples and doses employed in this study were 0.5, 1, 2, 3, and 4 kGy.

Table 1.Electron beam irradiation condition

Irradiation dose (kGy)	Conveyor velocity (m/min)	Dose rate (kGy/s)	Beam current (mA)
0.5	6.02	0.86	0.2
1	7.51	2.15	0.5
2	5.16	2.95	0.7
3	4.91	4.21	1
4	3.49	3.99	1

Microbial analysis

After irradiation, each sample (5 g) was blended with 45 mL of sterile saline solution and serially diluted using the sterile saline. Each diluent (0.1 mL) was spread on tryptic soy agar and tryptic soy agar containing 0.6% yeast extract were used for *E. coli* O157:H7 and *L. monocytogenes* respectively. Plates were incubated at 37°C for 48 h, and microbial counts were expressed as colony forming units per gram (CFU/g). Radiation sensitivity of the pathogens was calculated as D_{10}-value (the exposure kGy required to inactivate 90% of a population) using the following equation.

$$\mathrm{Log}N/N_0 = -k/D$$

k = kGy

N = the number of colonies per unit volume at k

N_0 = the number of colonies per unit volume at the k_0 (k_0 = 0 kGy)

Statistical analyses

Data was analyzed using SAS software (Release 8.01, SAS Institute, Inc., Cary, NC, USA). Statistical analysis was performed by one-way analysis of variance (ANOVA). The differences among the mean values were determined by the Duncan's multiple comparison tests at a confidence level of 95%.

III. RESULTS AND DISCUSSION

The bactericidal effect of EB irradiation on beef intestine is shown in Table 2. The initial number of *E. coli* O157:H7 and *L. monocytogenes* in small and large intestine were both 9.05 to 9.68 Log CFU/g in VP and AP. The number of pathogens decreased with the increase in irradiation dose. Furthermore, no viable cells were detected at an irradiation dose of 3 kGy in AP and 4 kGy in VP.

Irradiation induces water radiolysis and forms ions, free radicals, and reactive oxygen species (ROS) [6]. Free radicals could be peroxides and other ROS, which affect membranes and DNA of microorganisms through the further reactions with oxygen and these have ability to break chemical bond. Therefore, it is possible to attack polyunsaturated fatty acids in cell membranes or damage the DNA in microorganisms [9].

Table 2. Effect of electron-beam irradiation on the reduction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* (Log CFU/g) of beef intestines

Pathogens	Irradiation dose (kGy)	Small intestine		Large intestine	
		AP	VP	AP	VP
Escherichia coli O157:H7	0	9.68^{a}	9.57^{a}	9.05^{a}	9.46^{a}
	0.5	6.68^{b}	5.65^{b}	4.89^{b}	7.04^{b}
	1	4.85^{c}	4.83^{c}	3.47^{c}	5.86^{c}
	2	2.34^{d}	3.54^{d}	2.59^{d}	3.78^{d}
	3	ND^{e*}	2.05^{e}	ND^{e}	2.63^{e}
	4	ND^{e}	ND^{f}	ND^{e}	ND^{f}
	SEM[1]	0.080	0.057	0.145	0.100
Listeria monocytogenes	0	9.59^{a}	9.89^{a}	9.53^{a}	9.68^{a}
	0.5	6.92^{b}	6.31^{b}	4.56^{b}	6.86^{b}
	1	5.35^{c}	4.10^{c}	3.37^{c}	5.85^{c}
	2	3.02^{d}	3.32^{d}	2.38^{d}	3.86^{d}
	3	ND ^{e}	2.54^{e}	ND^{e}	2.27^{e}
	4	ND ^{e}	ND^{f}	ND^{e}	ND^{f}
	SEM[1]	0.074	0.119	0.056	0.063

[*]Viable with no growth at a detection limit < 10^{1} CFU/g.

[a-e]Values with different letters within the same column differ significantly (p<0.05).

[1] Standard errors of the mean (n=18).

The calculated D_{10}-value was higher for beef intestines with VP than those with AP (p<0.05) (Table 3.). The difference of D_{10}-value between aerobic and vacuum condition could be due to the oxygen permeability, since oxygen helps to form ROS easily.

S. Typhimurium inoculated in deboned chicken was more sensitive with aerobic packaging compared to vacuum packaging [7]. Thayer et al. [8] demonstrated that EB-irradiated beef patties packed in the lowest oxygen permeability package

had greater D_{10}-value than those packed under other packaging conditions.

Table 3. D_{10}-values (kGy) for different pathogens inoculated in beef intestines

Byproduct	Pathogen	Package		SEM[1]
		AP	VP	
Small intestine	*E. coli* O157:H7	0.33[b]	0.50[a]	0.006
	L. monocytogenes	0.33[b]	0.49[a]	0.008
	SEM[2]	0.008	0.006	
Large intestine	*E. coli* O157:H7	0.39[b]	0.47[a]	0.007
	L. monocytogenes	0.38[b]	0.46[a]	0.006
	SEM[2]	0.006	0.009	

[a,b]Values with different letters within the same row differ significantly (p<0.05).
[1]Standard errors of the mean (n=9), [2](n=6).

There were no significant differences in D_{10}-value between *E. coli* O157:H7 (Gram-negative) and *L. monocytogenes* (Gram-positive) inoculated on the beef intestines (Table 3). Thayer et al. [8] reported that the radiation D_{10}-value for *E. coli* O157:H7 and *L. monocytogenes* were not significantly different on beef, lamb, pork, and turkey (P>0.05). Jo et al. [10] explained that it is likely because some constituents of complex food system can affect the irradiation effect. However, in several studies it has been reported that Gram-positive and Gram-negative bacteria were affected differently by EB irradiation. In fermented oyster, D_{10}-values for *L. monocytogenes* (Gram-positive) and *Vibrio parahaemolyticus* (Gram-negative) were 0.60 and 0.29 kGy for gamma irradiation and 0.69 and 0.29 kGy for EB, respectively [11]. These differences are attributed to the structure of bacteria as peptidoglycan comprises 90% of the cell wall in gram-positive bacteria while in gram-negative bacteria it comprises 10% of the cell wall [12].

IV. CONCLUSION

Findings of the present study suggest that using EB irradiation makes complete inactivation of pathogens in small and large intestine of beef at doses of 3 and 4 kGy with AP and VP. The D_{10}-values with AP were lower than that of VP. The present study indicates that low-dose EB irradiation with AP can reduce the risk of contamination of meat byproducts by foodborne pathogens.

ACKNOWLEDGEMENTS

This work was supported from Radiation Technology R&D program (2013M2A2A6043308) through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning and Institute of Green Bio Science and Technology, Seoul National University, Republic of Korea.

REFERENCES

1. Toldrá, F., Aristoy, M. C., Mora, L., Reig, M. (2012). Innovations in value-addition of edible meat by-products. Meat Science 92: 290-296.
2. Álavarez-Astorgam, N., Capita, R., Alonso-Calleja, C., Moreno, B., Fernández, M.C. (2002). Microbiological quality of retail chicken by-products in Spain. Meat Science. 62: 45-50.
3. Devatkal, S., Mendiratta, S. K., Kondaiah, N., Sharma, M. C., Anjaneyulu, A. S. R. (2004). Physicochemical, functional and microbiological quality of buffalo liver. Meat Science. 68: 79-86.
4. Fabrizio, K. A., Sharma, R. R., Demirci, A., Cutter, C. N. (2002). Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce Salmonella species on poultry. Poultry Science. 81: 1598-1605.
5. Rivera, C. S., Blanco, D., Marco, P., Oria, R., Venturini, M. E. (2011). Effects of electron-beam irradiation on the shelf life, microbial populations and sensory characteristics of summer truffles (*Tuber aestivum*) packaged under modified atmospheres. Food Microbiology 28: 141-148.
6. Kim, H. J., Kang, M., Yong, H. I., Bae, Y. S., Jung, S., Jo, C. (2013). Synergistic effects of electron-beam irradiation and leek extract on the quality of pork jerky during ambient storage. Asian-Australas Journal of Animal Science 26: 596.
7. Thayer, D. W., Songprasertchap, S., Boyd, G. (1991). Effects of heat and ionizing radiation on *Salmonella* typhimurium in mechanically deboned chicken meat. Journal of Food Protection 54: 718-724.

8. Thayer, D. W., Boyd, G., Fox, J. B., Lakritz, L., Hampson, J. W. (1995). Variations in radiation sensitivity of foodborne pathogens associated with the suspending meat. Journal of Food Science 60: 63-67.
9. Harman, D. (1992). Free radical theory of aging. Mutation Research/DNAging. 275: 257-266.
10. Jo, C., Lee, N. Y., Kang, H. J., Shin, D. H., Byun, M. W. (2004). Inactivation of foodborne pathogens in marinated beef rib by ionizing radiation. Food Microbiology 21: 543-548.
11. Song, H. P., Kim, B., Jung, S., Choe, J. H., Yun, H., Kim, Y. J., Jo, C. (2009). Effect of gamma and electron beam irradiation on the survival of pathogens inoculated into salted, seasoned, and fermented oyster. Food Science and Technology 42: 1320-1324.
12. Atlas, R. M. (1995). Principles of Microbiology. St. Louis, (pp. 86–90). MO: Mosby-Year Book, Inc.