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Radiation sensitivity of foodborne pathogens in meat byproducts with different packaging

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HIGHLIGHTS

- Radiation sensitivities of pathogens in meat byproduct were tested.
- Electron beam irradiation of 3 or 4 kGy reduced pathogens by > 9 log
- The D_{10} values were lower in the aerobic-packaging than under vacuum condition.

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ABSTRACT

The aim of this study was to determine radiation sensitivity of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in edible meat byproducts. Seven beef byproducts (heart, liver, lung, lumen, omasum, large intestine, and small intestine) and four pork byproducts (heart, large intestine, liver, and small intestine) were used. Electron beam irradiation significantly reduced the numbers of pathogenic microorganisms in meat byproducts and no viable cells were detected in both aerobically- and vacuum-packaged samples irradiated at 4 kGy. Meat byproducts packed under vacuum had higher D_{10} value than the ones packed aerobically. No significant difference was observed between the D_{10} values of *E. coli* O157:H7 and *L. monocytogenes* inoculated in either aerobically or vacuum packaged samples. These results suggest that low-dose electron beam irradiation can significantly decrease microbial numbers and reduce the risk of meat byproduct contamination by the foodborne pathogens.

1. Introduction

Although muscle meat comprises a substantial portion of the livestock products, the edible meat byproducts, entrails, and internal organs has been widely consumed (Toldrá et al., 2012). Marti et al. (2011) reported that 40,000 pounds of various byproducts are produced and exported every month by the United States. Meat byproducts are inexpensive and their sensory and nutritional characteristics are distinct from the muscle meat. Some byproducts are also used in animal feed, cosmetics or medicine due to special components like minerals, vitamins, and hormones (Álavarez-Astorgam et al., 2002; Jayathilakan et al., 2012).

Meat byproducts may be contaminated with spoilage as well as pathogenic microorganisms due to unhygienic slaughtering and processing conditions. The pathogens like *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., and *Clostridium perfringens* could present notable hazards to humans and cause public health concerns (Devatkal et al., 2004; Tsola et al., 2008). These may originate from the digestive track of the animals or the environment of the slaughter house, even if chlorine, organic acids, or trisodium phosphate are used to control the growth of microorganisms (Álavarez-Astorgam et al., 2002; Devatkal et al., 2004; Fabrizio, et al., 2002). The only way to overcome these undesirable situations is through the implementation of hygienic processing, as there is no commercial non-thermal sterilization technology for meat byproducts.

Food irradiation can be used to increase the safety by reducing microbial growth and extending the shelf life of foods. Brazil, China, United States, United Kingdom, and most EU countries allow a larger number of irradiated foods (Rivera et al., 2011). The radiation used may be gamma ray, electron beam or X-ray. Gamma rays produced by radionuclides (^{60}Co or ^{137}Cs) have a high penetrating power, while electron-beams (EB) are produced from a

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machine source and have low but effective penetration. EB has advantages in food industry as it has lesser influence on the food quality and is consumer friendly due to non-use of radioisotopes (Rivera et al., 2011; Farkas, 1998). Several studies have shown that electron-beam irradiation significantly reduces the microbial counts in raw meats and meat products (Farkas, 1998; Kim et al., 2014; Park et al., 2010; Thayer et al., 1995). However, comparative information about the use of EB-irradiation on meat byproducts is still insufficient. Information about the bactericidal effect of EB-irradiation on meat byproducts is needed for consumers, suppliers, and health institutions to improve microbial safety of meat byproducts.

Therefore, the objective of this study was to determine radiation sensitivity of the foodborne pathogens *E. coli* O157:H7 and *L. monocytogenes* in beef and pork byproducts.

2. Materials and methods

2.1. Sample preparation and sterilization

Beef byproducts (heart, liver, lungs, lumen, omasum, large intestine, and small intestine) and pork byproducts (heart, large intestine, liver, and small intestine) were purchased from a livestock wholesale market in Daejeon and Gyeonggi, Korea. Prior to the inoculation test, each byproduct was vacuum packaged and sterilized by EB-irradiation (35 kGy at 10 MeV) using a linear electron beam RF accelerator (EB Tech, Daejeon, Korea) to achieve the complete inactivation of the indigenous microflora.

2.2. Test pathogens and culture condition

E. coli O157:H7 (KCCM 40406) and *L. monocytogenes* (KCTC 3569) were obtained from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) and Korean Collection for Type Culture (KCTC, Daejeon, Korea), respectively. *E. coli* O157:H7 and *L. monocytogenes* were cultivated in tryptic soy broth (Difco Laboratories, Detroit, MI, USA) and tryptic soy broth containing 0.6% yeast extract (Difco Laboratories), at 37 °C for 48 h. The cultures were then centrifuged at 3100 rpm for 15 min at 4 °C in a refrigerated centrifuge (UNION 32R, Hanil Science Industrial, Co., Ltd., Korea). The resulting pellet was washed twice with sterile saline solution (0.85%),the viable cell density was approximately 10^9 CFU/mL. Aliquots of 100 μL of the test culture preparation were inoculated on the cut beef and pork byproducts (5 g). Half the sample were vacuum packaged in low-density polyethylene/nylon vacuum bags (20 cm × 20 cm; oxygen permeability of 22.5 mL/m^2/24 h atm at 60% RH/25 °C; water vapor permeability of 4.7 g/m^2/24 h at 100% RH/25 °C) by a vacuum packaging machine (FJ-600XL; Hankook Fujee Industries Co., Hwaseong, Korea) at −650 mm Hg, and half were aerobically packaged in polyethylene bags (20 cm × 20 cm).

2.3. EB irradiation

Each prepared sample was irradiated on both sides in a linear EB RF accelerator (Energy 10 MeV, EB Tech., Daejeon, Korea) and conditions for irradiation are shown in Table 1. To confirm the target dose, alanine dosimeters, attached to the top and bottom surfaces of the sample packs, were read using a 104 Electron Paramagnetic Resonance unit (EMS-104; Bruker Instruments Inc., Bullerica, MA). The calculated maximum/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 conditions used for the present study.

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

2.4. Microbial analysis

After irradiation, each sample (5 g) was blended with 45 mL of sterile saline (0.85%) solution and serially diluted in sterile saline. Each diluent (0.1 mL) was spread on bacterial media, tryptic soy agar (Difco Laboratories) and tryptic soy agar containing 0.6% yeast extract (Difco Laboratories) 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).

2.5. Statistical analyses

The experiment was conducted as 3 independent trials with 3 observations for each treatment combination in each trial. 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 $p < 0.05$.

3. Results and discussion

3.1. Effect of packaging on growth of pathogens in EB-irradiated inoculated meat byproducts

The bactericidal effect of EB on seven kinds of beef byproducts is shown in Table 2. The initial populations of *E. coli* O157:H7 and *L. monocytogenes* were approximately 9.0 to 9.8 log CFU/g in both vacuum and aerobic packaging. These populations decreased significantly with increasing irradiation doses ($p < 0.05$). No viable cells were detected at an irradiation dose of 3 kGy in aerobic packaging and at a dose of 4 kGy in vacuum packaging except beef liver, omasum and pork large intestine. The electron beam-irradiated pork byproducts (heart, large intestine, liver, and small intestine) and beef byproducts (Table 3) showed similar trend under the two packaging conditions. The calculated D_{10} value (decimal reduction EB irradiation dose; the exposure kGy required to inactivate 90% of a population) was higher for vacuum packaged meat byproducts than the aerobically packaged ones ($p < 0.05$). *L. monocytogenes* inoculated in beef liver and pork large intestine showed no significant differences for packaging conditions (Tables 4 and 5).

Irradiation breaks chemical bonds, induces water radiolysis and forms ions, free radicals, and reactive oxygen species (ROS) (Kim et al., 2013; Reyes and Cisneros-Zevallos, 2007). Free radicals can directly attack polyunsaturated fatty acids in cell membranes or damage DNA in microorganisms. Hydroxyl radicals react on hydrogen atom attached to the carbon next to the double bonds of heterocyclic DNA. Hydrated electrons and H atoms derived from water by irradiation also react with the double bonds of pyrimidines in DNA and radical anions (electron adducts) are generated, which subsequently protonate in water (Dizdaroglu et al.,

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

Pathogens	Irradiation dose (kGy)	Heart		Lung		Large intestine		Liver	
		Aerobic	Vacuum	Aerobic	Vacuum	Aerobic	Vacuum	Aerobic	Vacuum
Escherichia coli O157:H7	0	9.62[a]	9.59[a]	9.26[a]	9.40[a]	9.05[a]	9.46[a]	9.60[a]	9.49[a]
	0.5	5.54[b]	6.55[b]	5.26[b]	7.15[b]	4.89[b]	7.04[b]	5.91[b]	6.91[b]
	1	3.90[c]	5.57[c]	3.99[c]	6.11[c]	3.47[c]	5.86[c]	4.50[c]	5.83[c]
	2	2.01[d]	4.58[d]	2.03[d]	3.88[d]	2.59[d]	3.78[d]	2.66[d]	3.74[d]
	3	ND[e*]	2.11[e]	ND[e]	2.47[e]	ND[e]	2.63[e]	ND[e]	2.58[e]
	4	ND[e]	ND[f]	ND[e]	ND[f]	ND[e]	ND[f]	ND[e]	ND[f]
	SEM[1]	0.074	0.067	0.066	0.033	0.145	0.100	0.092	0.088
Listeria monocytogenes	0	9.47[a]	9.79[a]	9.50[a]	9.75[a]	9.53[a]	9.68[a]	9.58[a]	9.53[a]
	0.5	5.42[b]	6.18[b]	5.63[b]	6.50[b]	4.56[b]	6.86[b]	6.88[b]	6.98[b]
	1	3.79[c]	5.36[c]	4.24[c]	5.60[c]	3.37[c]	5.85[c]	5.53[c]	5.82[c]
	2	1.73[d]	3.79[d]	3.06[d]	3.58[d]	2.38[d]	3.86[d]	2.98[d]	3.73[d]
	3	ND[e]	2.06[e]	ND[e]	2.36[e]	ND[e]	2.27[e]	1.02[e]	2.34[e]
	4	ND[e]	ND[f]	ND[e]	ND[f]	ND[e]	ND[f]	ND[f]	ND[f]
	SEM[1]	0.108	0.052	0.018	0.097	0.056	0.063	0.115	0.081

Pathogens	Irradiation dose (kGy)	Omasum		Rumen		Small intestine	
		Aerobic	Vacuum	Aerobic	Vacuum	Aerobic	Vacuum
Escherichia coli O157:H7	0	9.69[a]	9.22[a]	9.56[a]	9.39[a]	9.68[a]	9.57[a]
	0.5	6.79[b]	6.65[b]	6.77[b]	5.53[b]	6.68[b]	5.65[b]
	1	4.82[c]	4.54[c]	5.04[c]	4.86[c]	4.85[c]	4.83[c]
	2	3.45[d]	3.43[d]	2.80[d]	3.99[d]	2.34[d]	3.54[d]
	3	1.02[e]	2.95[e]	ND[e]	2.43[e]	ND[e]	2.05[e]
	4	ND[e]	ND[f]	ND[e]	ND[f]	ND[e]	ND[f]
	SEM[1]	0.082	0.139	0.073	0.116	0.080	0.057
Listeria monocytogenes	0	9.08[a]	9.11[a]	9.83[a]	9.67[a]	9.59[a]	9.89[a]
	0.5	6.15[b]	6.07[b]	6.02[b]	6.31[b]	6.92[b]	6.31[b]
	1	4.79[c]	4.72[c]	4.13[c]	4.10[c]	5.35[c]	4.10[c]
	2	2.19[d]	3.49[d]	2.59[d]	3.69[d]	3.02[d]	3.32[d]
	3	1.16[e]	2.58[e]	ND[e]	2.83[e]	ND[e]	2.54[e]
	4	ND[e]	ND[f]	ND[e]	ND[f]	ND[e]	ND[f]
	SEM[1]	0.072	0.058	0.066	0.099	0.074	0.119

[a–f]Values with different letters within the same column differ significantly ($p < 0.05$).

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

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

Table 3
Effect of electron-beam irradiation on the reduction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* (log CFU/g) of pork byproduct.

Pathogens	Irradiation dose (kGy)	Heart		Large intestine		Liver		Small intestine	
		Aerobic	Vacuum	Aerobic	Vacuum	Aerobic	Vacuum	Aerobic	Vacuum
Escherichia coli O157:H7	0	8.64[a]	8.94[a]	9.73[a]	9.51[a]	9.07[a]	9.63[a]	9.30[a]	9.38[a]
	0.5	4.58[b]	6.93	4.69	6.31	7.02	5.54	4.74	6.84
	1	4.04[c]	6.11[c]	3.30[c]	5.08[c]	4.25[c]	4.44[c]	3.14[c]	5.67[c]
	2	1.56[d]	2.67[d]	2.16[d]	3.52[d]	2.34[d]	2.44[d]	1.80[d]	3.09[d]
	3	ND[e*]	1.79[e]	ND[e]	2.28[e]	ND[e]	1.92[e]	ND[e]	1.49[e]
	4	ND[e]	ND[f]	ND[e]	ND[f]	ND[e]	ND[f]	ND[e]	ND[f]
	SEM[1]	0.145	0.067	0.086	0.087	0.180	0.115	0.076	0.066
Listeria monocytogenes	0	8.86[a]	8.63[a]	9.54[a]	9.64[a]	9.20[a]	9.38[a]	9.58[a]	9.38[a]
	0.5	4.74[b]	6.54[b]	6.89[b]	6.71[b]	7.37[b]	6.45[b]	4.52[b]	6.72[b]
	1	3.33[c]	6.12[c]	5.21[c]	5.37[c]	5.00[c]	4.11[c]	3.51[c]	5.64[c]
	2	2.10[d]	2.33[d]	3.02[d]	3.62[d]	2.70[d]	2.78[d]	1.76[d]	3.22[d]
	3	ND[e]	1.64[e]	1.12[e]	2.10[e]	ND[e]	1.92[e]	ND[e]	2.01[e]
	4	ND[e]	ND[e]	ND[e]	ND[f]	ND[e]	ND[f]	ND[e]	ND[f]
	SEM[1]	0.010	0.168	0.101	0.045	0.255	0.072	0.127	0.061

[a–f]Values with different letters within the same column differ significantly ($p < 0.05$).
[*]Viable with no growth at a detection limit $< 10^1$ CFU/g.
[1] Standard errors of the mean ($n = 18$).

Table 4
D_{10} values (kGy) for different pathogens inoculated in beef byproducts.

	Pathogen	Package		SEM[1]
		Aerobic	Vacuum	
Heart	*E. coli* O157:H7	0.34[b]	0.47[a]	0.007
	L. monocytogenes	0.35[b]	0.43[a]	0.009
	SEM[2]	0.006	0.010	
Lung	*E. coli* O157:H7	0.36[b]	0.46[a]	0.008
	L. monocytogenes	0.36[b]	0.47[a]	0.009
	SEM[2]	0.011	0.003	
Large intestine	*E. coli* O157:H7	0.39[b]	0.47[a]	0.007
	L. monocytogenes	0.38b[b]	0.46[a]	0.006
	SEM[2]	0.006	0.009	
Liver	*E. coli* O157:H7	0.35[by]	0.47[a]	0.008
	L. monocytogenes	0.43[x]	0.46	0.012
	SEM[2]	0.013	0.007	
Omasum	*E. coli* O157:H7	0.44[b]	0.51[a] >	0.015
	L. monocytogenes	0.47[b]	0.52[a]	0.007
	SEM[2]	0.020	0.003	
Rumen	*E. coli* O157:H7	0.33[b]	0.52[a]	0.008
	L. monocytogenes	0.34[b]	0.51[a]	0.007
	SEM[2]	0.009	0.006	
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	

[a,b] Values with different letters within the same row differ significantly ($p < 0.05$).
[x,y] Values with different letters within the same column differ significantly ($p < 0.05$).

[1] Standard errors of the mean ($n = 6$).
[2] ($n = 6$).

Table 5
D_{10} values (kGy) for different pathogens inoculated in pork byproducts.

	Pathogen	Package		SEM[1]
		Aerobic	Vacuum	
Heart	*E. coli* O157:H7	0.39[b]	0.46[a]	0.008
	L. monocytogenes	0.39[b]	0.46[a]	0.012
	SEM[2]	0.010	0.007	
Large intestine	*E. coli* O157:H7	0.37[by]	0.48[a]	0.009
	L. monocytogenes	0.44[x]	0.46	0.004
	SEM[2]	0.009	0.00	
Liver	*E. coli* O157:H7	0.34[b]	0.49[a]	0.011
	L. monocytogenes	0.33[b]	0.49[a]	0.005
	SEM[2]	0.006	0.010	
Small intestine	*E. coli* O157:H7	0.37[b]	0.45[a]	0.008
	L. monocytogenes	0.37[b]	0.46[a]	0.007
	SEM[2]	0.008	0.007	

[a,b] Values with different letters within the same row differ significantly ($p < 0.05$).
[x,y] Values with different letters within the same column differ significantly ($p < 0.05$).

[1] Standard errors of the mean ($n = 6$).
[2] ($n = 6$).

2002; Oleinick et al., 1987). The free radicals could undergo further reactions, e.g., addition of oxygen leading to the formation of peroxides and other ROS, which affect membranes and DNA of microorganisms (Harman, 1992).

Several studies reported that irradiation dose, processing temperature, and packaging conditions strongly influence the microbiology and quality of irradiated foods (Ahn et al., 1998; Farkas, 1998; Jo et al., 2004). *Pseudomonas putida*, *E. coli*, and *Moraxella phenylpyruvica* inoculated in minced chicken meat and *S. typhimurium* inoculated in deboned chicken were more sensitive when irradiated in aerobic conditions than under vacuum packaging (Patterson, 1988; Thayer et al., 1991). Murano et al. (1999) proposed that packaging under vacuum reduces the number of oxygen radicals and reactive oxygen species formed during irradiation. Similarly, Thayer et al. (1995) reported that EB-irradiated beef patties packed in the lowest oxygen permeability package had greater D_{10} than those packed under other packaging conditions. The increased D_{10} could be due to the low oxygen permeability,

which makes it difficult for oxygen-derived radicals to migrate easily.

3.2. Effect of EB-irradiation on growth of pathogens in inoculated meat byproducts

Several studies revealed that Gram-positive and Gram-negative bacteria were affected differently by EB-irradiation. Kang et al. (2012) showed that *S. typhimurium* (Gram-negative) inoculated in pork jerky was sensitive to EB-irradiation combined with treatment with leek extract, while *L. monocytogenes* (Gram-positive) did not show sensitivity under the same conditions. Song et al. (2009) reported that in fermented oyster, D_{10} values for *L. monocytogenes* 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. These differences in radiation sensitivity are attributed to the structural differences of these bacteria. Gram-negative bacteria have hydrophilic cell walls consisting of lipopolysaccharides, whereas cell walls of Gram-positive bacteria mainly contain a thick layer of a unique peptidoglycan that is important for their survival (Nikaido, 1996). In case of *Staphylococcus aureus*, staphyloxanthin, a membrane-bound carotenoid acts as a radical scavenger to prevent the damage by reactive oxygen species and resulting in a relatively high resistance to radiation (Clauditz et al., 2006).

In the present study, no significant differences in D_{10} values of *E. coli* O157:H7 and *L. monocytogenes* inoculated in the meat byproducts were observed except in aerobically packaged beef liver (Tables 4 and 5). Thayer et al. (1995) reported that the radiation D_{10} for *E. coli* O157:H7 and *L. monocytogenes* were not significantly ($P < 0.05$) different on beef, lamb, pork, and turkey. Also, the D_{10} of *L. monocytogenes*, *E. coli*, and *S. typhimurium* observed in the pork jerky combined with onion peel extract and barbecue flavor were 0.19, 0.18, and 0.19 kGy, respectively (Kim et al., 2014). However, a further study for confirmation is needed due to the fact that the majority of previous studies display stronger resistance in Gram-positive than Gram-negative bacteria (Lung et al., 2015).

4. Conclusion

Electron beam irradiation significantly reduced the numbers of inoculated *E. coli* O157:H7 and *L. monocytogenes* in meat byproducts and no viable cells were detected in both aerobically- and vacuum-packaged samples irradiated at 4 kGy. The D_{10} values of pathogens were lower in the aerobic packaging than under vacuum-packaging conditions. The present study indicates that low-dose electron beam irradiation can reduce the risk of contamination of meat byproducts by foodborne pathogens.

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