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RESEARCH NOTE

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Comparison on Inactivation of *Enterobacter sakazakii, Salmonella typhimurium*, and *Bacillus cereus* Inoculated on Infant Formula During Storage by Gamma Irradiation

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Abstract *Enterobacter sakazakii, Salmonella typhimurium,* and *Bacillus cereus* were evaluated on inoculated infant formula by gamma irradiation treatment as a method to provide microbial safety. The infant formula inoculated with the major pathogenic bacteria was treated at irradiation dose of 0, 3, 5, and 10 kGy, respectively. After treatment, the samples were individually packaged and stored at 20°C. Microbiological data during storage represented that the populations of *E. sakazakii, S. typhimurium,* and *B. cereus* were reduced with the increase of irradiation dose by 4 to 5 log reductions. In particular, *E. sakazakii, S. typhimurium,* and *B. cereus* were eliminated at 10, 5, and 3 kGy, respectively. *E. sakazakii* was the most radiation-resistant, while *B. cereus* was the least. Our results represent that gamma irradiation below 10 kGy should eliminate the growth of the major pathogenic bacteria in infant formula during storage.

Keywords: infant formula, gamma irradiation, pathogenic bacteria, storage

Introduction

Enterobacter sakazakii, Bacillus cereus, and *Salmonella typhimurium* are among the major pathogenic bacteria that have been associated with food poisoning in infant foods. In particular, *E. sakazakii* in infant formula has been implicated in infections among the high-risk infants (1,2). Necrotizing enterocolitis and meningitis caused by *E. sakazakii* have high mortality rates (3,4). In addition, dried milk products are known to be easily contaminated with *B. cereus* spores (5), causing the foodborne diseases such as diarrheal and emetic syndromes (6,7).

Infant formula is classified into special nutrient food, and it should have hygienic safety. As a food preservation method, ionizing irradiation is considered an effective method to provide food with hygienic quality by reducing the microbial spoilage. There are two types of ionizing radiation; gamma ray and electron beam. Gamma irradiation is recognized throughout the world as a means of reducing microbial spoilage (8).

There have been a few studies on the inactivation of *E. sakazakii* in dehydrated infant formula by gamma irradiation (9-11). However, those studies have been mainly focused on the calculation of D-value for the inactivation of *E. sakazakii*. In addition, it should be noted that there are other pathogenic bacteria concerned regarding the possibility of bacterial contamination in manufacturing process of infant formula. However, inactivation on the major pathogenic bacteria such as *B. cereus* and *S. typhimurium* which are more probable bacteria contaminated in infant formula was not studied.

Therefore, the objective of this study was to compare the effect of gamma irradiation treatment to inactivate the major pathogenic bacteria such as *B. cereus, S. typhimurium as well as E. sakazakii* inoculated on infant formula during storage at 20°C.

Materials and Methods

Materials Infant formula (Step Myungpoom, age of 6-9 month; Namyang Co., Gongju, Korea) was purchased from a local market in Daejeon, Korea. The sample was not detected to be contaminated with the pathogenic bacteria studied in this study and had 3.5% water content.

Culture conditions *E. sakazakii* (ATCC 51329), *B. cereus* (KCCM 40935), and *S. typhimurium* (ATCC 14028) cultures were grown at 37°C for 24 hr in 50-mL tubes containing 25 mL of Enterobacteriaceae enrichment broth (Oxoid, Basingstoke, UK), brain heart infusion (BHI) (Oxoid), and Salmosyst (Merck, Darmseadt, Germany), respectively.

Inoculation on infant formula Powdered infant formula (10 g) was placed into a sterile polyethylene (PE) bag and inoculated with 200 μL of *E. sakazakii, B. cereus,* and *S. typhimurium* to obtain an initial level of 10^5 CFU/g, respectively. After inoculation, the samples were shaken

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for 1 min to evenly spread the bacteria on the infant formula. Samples were then individually packaged in 100 ×170 mm low density polyethylene (LDPE) bags.

Irradiation treatments Samples were irradiated using a ^{60}Co gamma ray irradiator (Type IR-79; Nordion International Inc., ON, Canada) at the Korea Atomic Energy Research Institute (KAERI, Jeongeup, Korea) with 3, 5, and 10 kGy. Source strength was 100 kCi and the dose rate was 10 kGy/hr. Dosage was determined using a 5 mm diameter alanine dosimeter (Bruker Instruments, Rheinstetten, Germany). The dosimeter was calibrated against an international standard set by the International Atomic Energy Agency (IAEA, Vienna, Austria). After irradiation, samples were stored at 20°C for 9 days.

Microbiological analysis After gamma irradiation, the samples (10 g) were removed using a sterile scalpel. The samples were then homogenized using a stomacher (MIX 2; AES Laboratoire, France) for 3 min, and diluted with peptone water (0.1% sterile peptone, w/v) for microbial count. Serial dilutions were performed in triplicate on each selective agar plate. *E. sakazakii* counts were determined by plating appropriately diluted samples onto *E. sakazakii* agar (Oxoid). Samples were evenly spread on the surface of the plates with a sterile glass rod. *S. typhimurium* were plated onto xylose lysine deoxychlorate (XLD; Difco Laboratoires, Detroit, MI, USA). *B. cereus* counts were determined by plating appropriately diluted samples onto mannitol egg yolk polymyxin (MYP; Oxoid). All plates were incubated at 37°C for 24 hr. Each experiment was repeated 3 times and microbial count was the mean of 3 determinations. Microbial counts were expressed as log CFU/g.

Results and Discussion

The initial populations of *E. sakazakii*, *S. typhimurium*, and *B. cereus* in the infant formula after inoculation were 4.68, 4.90, and 4.19 log CFU/g, respectively. Gamma irradiation treatment decreased significantly the populations in *E. sakazakii*, *S. typhimurium*, and *B. cereus* on infant formula, compared to the control (Fig. 1). Increase of radiation dose decreased the microbial populations. In particular, *E. sakazakii*, *S. typhimurium*, and *B. cereus* were eliminated at 10, 5, and 3 kGy, respectively. Among the major pathogenic bacteria inoculated on infant formula, *E. sakazakii* was the most radiation-resistant, while *B. cereus* was the least.

Only a few studies have been reported on the effect of ionizing irradiation on infant formula. Osaili *et al.* (9) have reported that the populations of *E. sakazakii* in dehydrated infant formula were decreased by 3 log cycle at 5.13 kGy. There were also reports on the effect of gamma irradiation on dehydrated infant formula, resulting in complete elimination of *E. sakazakii* at 5 kGy (10,11). Our results are in sharp contrast with those reports (10,11), since *E. sakazakii* was not inactivated at 5 kGy in our study. This difference can be attributed to the different experimental methods on inoculation as well as different strain and storage condition. Our study was reflected on the actual circumstances where pathogenic bacteria could be incorporated into infant formula during manufacturing process.

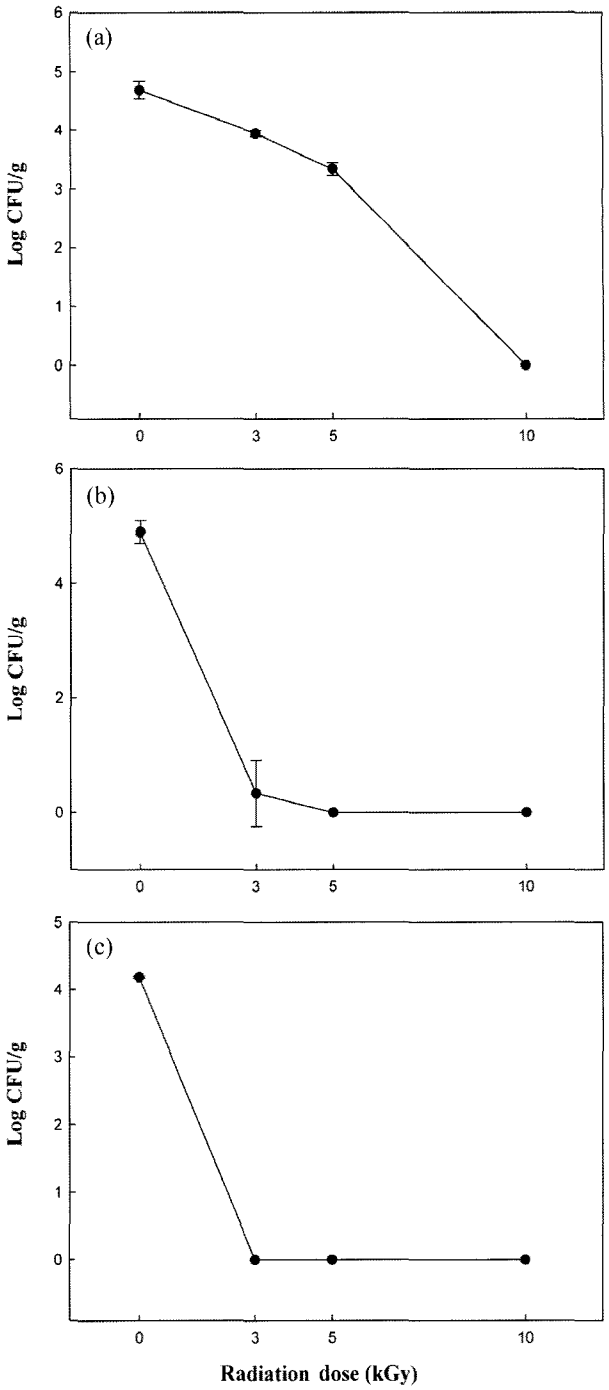

Fig. 1. Effect of gamma irradiation on the survival of the pathogenic bacteria inoculated on infant formula. Bars represent standard error. (a) *Enterobacter sakazakii*, (b) *Salmonella typhimurium*, (c) *Bacillus cereus*.

After gamma irradiation treatment, the populations of *E. sakazakii* in infant formula decreased to 3.95 and 3.35 log CFU/g at 3 and 5 kGy, respectively (Fig. 1a). The population of *E. sakazakii* was not detected above 10 kGy. After 9 days of storage, the control had 4.14 log CFU/g, while the populations of *E. sakazakii* for the samples treated with 3 and 5 kGy had 2.66 and 1.82 log CFU/g, respectively (Fig. 2a). These results indicate that the effect of gamma irradiation on the microbial inactivation is in effect during storage.

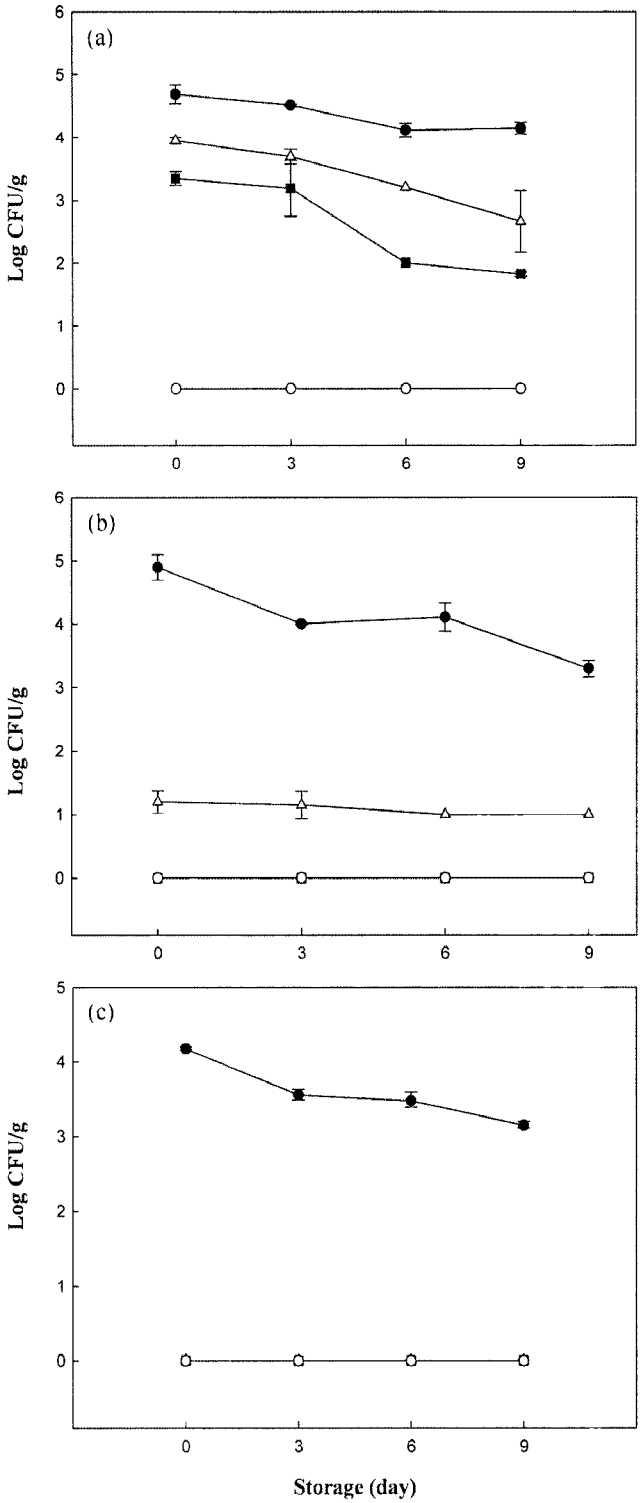

Fig. 2. Effect of gamma irradiation on the growth of the pathogenic bacteria inoculated on infant formula during storage. Bars represent standard error. (a) *Enterobacter sakazakii*, (b) *Salmonella typhimurium*, (c) *Bacillus cereus*. ●, Control; △, 3 kGy; ■, 5 kGy; ○, 10 kGy.

For *S. typhimurium*, irradiation at 3 kGy decreased the populations from 4.90 to 1.20 log CFU/g, resulting in 3.7 log reduction (Fig. 1b). In addition, irradiation above 5 kGy completely eliminated *S. typhimurium*. Martins *et al.* (12) have reported that gamma irradiation at 1.7 kGy reduced the populations of *Salmonella* spp. in watercress by 4 log cycle. Kwon and Waje (13) have also reported that *Salmonella* in alfalfa sprouts fell below detection limits (1 log CFU/g) by irradiation above 0.5 kGy. During storage, the populations of *S. typhimurium* for the non-irradiated decreased to 3.30 log CFU/g after 9 days, while the irradiated at 3 kGy reached 1.0 log CFU/g (Fig. 2b). The decrease during storage can be explained by the microbial growth condition of low water activity in the infant formula.

Among the pathogenic bacteria used in this study, *B. cereus* was the most sensitive to gamma irradiation. The populations of *B. cereus* were eliminated completely by gamma irradiation above 3 kGy (Fig. 1c). Sarrias *et al.* (14) have reported that *B. cereus* contamination in raw rice was eliminated by radiation at 7.5 kGy. Our results are comparable with the report. After 9 days of storage, the control had 3.15 log CFU/g, while the populations of *B. cereus* for the samples treated with gamma irradiation were not detected (Fig. 2c). Therefore, our results clearly indicate that gamma irradiation is an efficient way of inactivating the major foodborne pathogens in infant formula.

FAO/IAEA/WHO Expert Committee on Food Irradiation (JECFI) recommends 10 kGy as an upper dose for food irradiation processes, which is considered to be safe, meaning that there is no toxicological evidence at this dose (15). Our study showed that gamma irradiation at 10 kGy eliminated the major pathogenic bacteria such as *E. sakazakii*, *S. typhimurium*, and *B. cereus* in infant formula during storage up to 9 days, securing the microbial safety.

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