Research Note

Gamma Radiation Sensitivity of Enterobacter sakazakii in Dehydrated Powdered Infant Formula

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ABSTRACT

The observed Enterobacter sakazakii D10-values for tryptic soy broth and dehydrated powdered infant formula were 0.27 ± 0.05 and 0.76 ± 0.08 kGy, respectively. A decrease of approximately 3 log in the dehydrated powdered infant formula was obtained by irradiation with 3.0 kGy or rehydration with hot water at 80°C. No recoverable bacteria were found in the powdered infant formula irradiated at 5.0 kGy and stored, either before or after rehydration. A radiation dose of up to 5.0 kGy had no marked effect on the sensory properties of the dehydrated powdered infant formula after rehydration and heating. Gamma radiation could potentially be used to inactivate E. sakazakii in dehydrated powdered infant formula; however, nutritional studies need to be conducted before the use of radiation can be recommended.

The objectives of this study were to evaluate the inactivation effects of a gamma radiation on E. sakazakii, to determine (i) the radiation sensitivity of E. sakazakii in a microbiological broth medium and dehydrated powdered infant formula, (ii) the survival and potential for a growth of E. sakazakii on irradiated infant formula during a storage, and (iii) the effect of radiation doses on the sensory properties of a dehydrated powdered infant formula.

MATERIALS AND METHODS

Bacterial strain and media. E. sakazakii strain ATCC 29544 (KCTC 2949) used in this study was obtained from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea). The original source of the strain is a sample taken from a child’s throat at a hospital laboratory. The strain was cultured in 20 ml of sterilized tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) in a 100-ml flask at 37°C for 2 h with agitation (150 rpm). Portions of the culture (0.1 ml) were transferred to 50 ml of new sterilized TSB in 250-ml flasks and grown for 18 h at 37°C while being agitated (150 rpm). They were then centrifuged (2,795 × g for 10 min at 4°C) in a refrigerated centrifuge (VS-5500, Vision Scientific Co., Seoul, Korea). The culture was washed twice with sterile buffered peptone water (Difco). The pellet was then suspended in sterile buffered peptone water to obtain a concentration of inoculum that was such that, when initially examined, there was approximately 105 CFU/ml when the formula was rehydrated. Enumeration of bacteria in the inoculum in a culture was determined by plating 0.1-ml portions of an appropriately diluted culture on duplicate tryptic soy agar (Difco) plates, followed by incubation at 37°C for 24 h.

Sample preparation and inoculation. A can of normal commercial dehydrated powdered infant formula (Mail Co. Ltd., Pyungteck, Korea) made from desalted cow’s milk designed as food for an age range of 3 to 5 months was purchased from a...
local supermarket. Total microorganisms in the formula was evaluated by enrichment test before inoculation. The enrichment test was independently duplicated. A 1:10 dilution of the powdered infant formula in buffered peptone water was incubated at 36°C overnight for preenrichment. A 10-ml aliquot of this culture was added to 90 ml of Enterobacteriaceae enrichment broth (CM0317, Oxoid, Basingstoke, UK) or TSBR, respectively, and incubated at 36°C overnight. Enriched broth was then spread on violet-red bile glucose agar (CM0485; Oxoid) or tryptic soy agar and incubated at 25°C for 48 to 72 h. No organisms detected on the powdered infant formula were used the present study. Fifty grams of the dehydrated powdered infant formula was inoculated and completely dissolved with 50 ml of the E. sakazakii ATCC 29544 inoculum. The wet, blended inoculation was lyophilized at −70°C. The infant formula inoculated with E. sakazakii was powdered in a sterile blender jar. The water activity (a_w) of the powdered infant formula was determined with Humidit-I (Novasina, Zurich, Switzerland) at 25°C. Calibration was first carried out at 25°C by using calibration salts of 11, 33, 53, 75, 90, and 98% relative humidity. The a_w of noninoculated formula immediately upon opening it was 0.17 ± 0.02, and that of the inoculated dehydrated powdered formula was 0.18 ± 0.01.

Radiation D_{10}-values for E. sakazakii. To determine D_{10}-values, the E. sakazakii culture in TSB was irradiated at 0.0 (control), 0.3, 0.5, 0.7, 1.0, 1.5, or 2.0 kGy. The dehydrated powdered infant formula inoculated with E. sakazakii ATCC 29544 was also irradiated with 0.0 (control), 1.0, 2.0, 3.0, or 4.0 kGy in its powdered state after lyophilization. The amount of surviving E. sakazakii was determined by plating 0.1-ml portions of an appropriately diluted sample on duplicate tryptic soy agar plates, then incubated at 37°C for 24 h. The D-value—the radiation dose (kGy) that produces a 90% reduction in numbers of viable cells—was determined by graphing the log number of E. sakazakii survivors per gram versus the radiation dose (kGy) (Excel 2003; Microsoft Corp., Redmond, Wash.) (21). The D-value was calculating by taking the negative reciprocal of the slope (3).

Gamma radiation. Samples were irradiated in a cobalt-60 irradiator (point source AECL, IR-79; MDS Nordion International Co. Ltd., Ottawa, Ontario, Canada) at the Korea Atomic Energy Research Institute, Daejeon. The source strength was approximately 100 kCi, with a dose rate of 10 kGy/h at 20°C. Dosimetry was performed with 5-mm-diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured with a Bruker EMS 104 EPR analyzer. The dosimeters were calibrated against an international standard set by the International Atomic Energy Agency (Vienna, Austria).

Determination of survival and growth. The inoculated dehydrated powdered infant formulas were placed in glass bottles. The bottles were sealed in an air-tight manner with poly(tetrafluoroethylene)-coated rubber septa and aluminum caps, and they were irradiated with 0.0 (control), 1.0, 3.0, or 5.0 kGy. In the present study, the accelerated storage test at 30°C was applied; it is possible to predict effect of radiation on microbial growth (16). The samples were incubated at 30°C and then tested at 0, 1, 3, 6, 10, and 15 days. For each test, the samples were tested after being rehydrated with 10× sterile distilled deionized water, then stored at 10°C for 0, 1, 3, and 6 h. The formula was rehydrated by sterile distilled deionized water at room temperature. In order to compare the survival bacterial population with the heat treatment, another unirradiated formula was rehydrated with hot sterile distilled deionized water at 80°C, then stored at 10°C after being cooled for 10 min at room temperature. The surviving populations of E. sakazakii were counted for each time stored at 10 or 30°C by plating directly or after serial dilutions on duplicate tryptic soy agar plates, followed by incubation at 30°C for 24 h.

Sensory analysis. For the intensity sensory analysis, the commercial dehydrated powdered infant formula was treated with 0.0 (control), 1.0, 3.0, or 5.0 kGy in a glass bottle. The samples were rehydrated according to the manufacturers’ instructions on the label, i.e., 200 ml of room-temperature distilled deionized water was added to 28.0 g of the dried formula in a 250-ml glass bottle with a screw cap. The intensity sensory analysis of the irradiated infant formulas were performed at two different temperatures (room temperature and 50°C) by an 11-member trained sensory panel. For the sensory analysis at 50°C, the rehydrated samples stored in screw-cap bottles were heated to an internal temperature of 50°C (temperature was monitored with thermocouple-equipped heat-resistant wire and probe) in a preequilibrated water bath to 50°C. A 7-point scale was used for an analysis of the intensity of the color (1, white; 7, brown) and flavor (1, very weak; 7, very strong) of the irradiated infant formula. In order to compare and calibrate, an unirradiated control sample served as a reference at each session. The samples were assigned random numbers, and their serving order was random for all the assessors. The collection of the sensory data was evaluated as a significance of the differences among the samples by Duncan’s multiple-range test at the 5% level (Windows SPSS 10.0; SPSS Inc., Chicago, Ill.).

RESULTS AND DISCUSSION

D_{10}-values. Radiation effectively reduced the population of E. sakazakii in both the TSB and dehydrated powdered infant formula (Fig. 1). The D_{10}-values for the TSB and dehydrated powdered infant formula were 0.27 and 0.76 kGy, respectively, as assessed by linear regression performed with all viable count data. The susceptibility of E. sakazakii ATCC 29544 to gamma radiation is similar to that of other gram-negative foodborne pathogens. D_{10}-values in the 0.2- to 0.5-kGy range are typically observed with broth cultures of gram-negative pathogens such as E. coli or Salmonella Typhimurium in broth (3).

The 0.27 ± 0.05 kGy D_{10}-value for E. sakazakii ATCC 29544 in TSB was based on inclusion of all data points. However, there was some indication of a shoulder: the inactivation was not completely linear at the lower radiation

FIGURE 1. Survival of E. sakazakii after gamma radiation of inoculated tryptic soy broth (A) and dehydrated powdered infant formula (B). Error bars represent standard error. Experiments were independently replicated three times.
TABLE 1. Survival and regrowth of E. sakazakii inoculated on infant formula after gamma radiation

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>Storage time after rehydration (h)</th>
<th>Gamma radiation (kGy)</th>
<th>Heat (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>8.51 ± 0.05</td>
<td>7.76 ± 0.12</td>
</tr>
<tr>
<td>3</td>
<td>8.60 ± 0.09</td>
<td>8.76 ± 0.01</td>
<td>5.88 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>8.59 ± 0.13</td>
<td>7.79 ± 0.05</td>
<td>5.04 ± 0.03</td>
</tr>
<tr>
<td>1</td>
<td>8.68 ± 0.08</td>
<td>7.40 ± 0.19</td>
<td>4.92 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>8.61 ± 0.19</td>
<td>7.34 ± 0.01</td>
<td>6.84 ± 0.09</td>
</tr>
<tr>
<td>6</td>
<td>8.74 ± 0.02</td>
<td>7.26 ± 0.02</td>
<td>4.94 ± 0.05</td>
</tr>
<tr>
<td>0</td>
<td>8.30 ± 0.02</td>
<td>6.79 ± 0.05</td>
<td>5.48 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>8.23 ± 0.15</td>
<td>6.79 ± 0.05</td>
<td>4.94 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td>8.15 ± 0.05</td>
<td>6.79 ± 0.05</td>
<td>4.94 ± 0.05</td>
</tr>
</tbody>
</table>

* Experiments were independently duplicated replicates. Values are enumeration of E. sakazakii ± SD.

* Dehydrated powdered infant formula at 30°C after gamma radiation.

* Rehydrated infant formula at 10°C.

* Dehydrated powdered infant formula was tested after rehydration by room temperature water.

* Dehydrated powdered infant formula was tested after rehydration by heated water at 50°C.

dose levels (Fig. 1). When the D<sub>10</sub>-value was recalculated excluding the 0.3-kGy dose values, the value was 0.22 kGy, with R² = 0.997.

The D<sub>10</sub>-values for the dehydrated powdered infant formula was substantially greater than the TSB. The sensitivity of the microorganisms to irradiation is affected by a variety of factors, such as temperature during an irradiation, stage of growth, presence of oxygen or antioxidants, availability of water, and composition of the medium (17). Typically the level of radiation needed to inactivate bacteria in dehydrated powdered products is increased as a result of the lack of available water, thus limiting secondary effects (indirect action) resulting from water radioisoly and limiting antimicrobial action to direct damage of the bacterial DNA (3).

This study determined the sensitivity of E. sakazakii ACTT 29544 by gamma radiation. However, further research is needed to assess radiation resistance among a variety of E. sakazakii isolates.

**Survival and regrowth.** Many investigators have recommended that dried infant formula be rehydrated with water at a temperature of 70°C or more to inactivate E. sakazakii (4, 6, 9, 14). Edelson-Mammel and Buchanan (4) reported on the ability of 12 strains of E. sakazakii to survive heating in a rehydrated infant formula heated with a submerged coil apparatus. The authors observed that $D_{85}$ values ranged from 30.5 to 591.9 s for the 12 strains, and the value was 367.1 ± 23.4 s for E. sakazakii ATCC 29544, a thermally resistant strain. In this study, the inactivation effect of E. sakazakii was compared between radiation and hot water (80°C). The mean initial population of E. sakazakii in control samples was 8.5 ± 0.05 log CFU/g. Gamma radiation with 3 kGy or a rehydration with hot water (80°C) reduced E. sakazakii population to 5.91 ± 0.03 and 5.40 ± 0.03 log CFU/g, respectively. Radiation with 5 kGy reduced E. sakazakii population to below the limit of detection (<10² CFU/g).

In a storage study, E. sakazakii inoculated on dehydrated powdered infant formula did not increase any more than the population at day 0 (Table 1). It may be due to a reason, despite the low contamination level, infections associated with E. sakazakii, therefore, it is important to eliminate E. sakazakii, especially in an infant formula.

Many reports have been concerned about the growth of E. sakazakii in reconstituted infant formula (5, 9, 14, 18). As found in a previous study, E. sakazakii strains generally grow at 6 to 45°C, while the temperatures of many refrigerators range from 7 to 10°C. These conditions would allow E. sakazakii to grow (14). In the present study, as a result of a storage study of the rehydrated infant formula at each storage time, we found that the bacteria did not grow until 6 h of storage at 10°C (Table 1). Iversen et al. (11) observed that the average lag time of E. sakazakii was
TABLE 2. Scores of sensory properties of irradiated infant formula after rehydration

<table>
<thead>
<tr>
<th>Temp</th>
<th>Dose (kGy)</th>
<th>Color</th>
<th>Milk flavor</th>
<th>Off flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temp</td>
<td>0</td>
<td>4.67 ± 1.00c</td>
<td>4.11 ± 0.601c</td>
<td>1.89 ± 0.928c</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.11 ± 0.928</td>
<td>4.22 ± 0.833</td>
<td>1.78 ± 1.301</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.11 ± 0.781</td>
<td>4.56 ± 1.014</td>
<td>1.67 ± 0.707</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.22 ± 0.833</td>
<td>4.33 ± 0.866</td>
<td>1.89 ± 0.928</td>
</tr>
<tr>
<td>Heated at 50°C</td>
<td>0</td>
<td>4.00 ± 0.707c</td>
<td>4.00 ± 0.866c</td>
<td>1.33 ± 0.707c</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.33 ± 1.118</td>
<td>3.78 ± 0.972</td>
<td>1.56 ± 1.013</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.78 ± 0.600</td>
<td>4.11 ± 0.928</td>
<td>1.89 ± 1.054</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.11 ± 0.601</td>
<td>4.11 ± 1.054</td>
<td>2.11 ± 0.782</td>
</tr>
</tbody>
</table>

a Experiments were independently duplicated replicates. Values are intensity score ± SD.

b Dehydrated powdered infant formula was tested after rehydration by room temperature water. Dehydrated powdered infant formula was tested after rehydration and heating at 50°C.

c Not significantly different among the samples by Duncan’s multiple range test at a 5% level.

2 h, and the average doubling time was 13.6 h at 10°C. In order to reduce the health risk, everything must be done to effectively control E. sakazakii levels in dehydrated powdered infant formula (5, 14).

Gamma radiation up to 5.0 kGy had no marked effect on the color and flavor of the dehydrated powdered infant formula after rehydration and heating (Table 2).

In summary, gamma radiation at a dose of 5 kGy eliminated E. sakazakii inoculated at 8.0 to 9.0 log CFU/g onto a dehydrated powdered infant formula without affecting its sensory properties. However, considering the observed very low contamination levels (1 to 10 CFU/100 g) of E. sakazakii in the dehydrated powdered infant formula, an inactivation dose must be established to assure the formula’s safety and to minimize the loss of nutritional properties.

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REFERENCES


