

## Application of Gamma Irradiation for Inactivation of *Enterobacter sakazakii* in Dehydrated Infant Formula

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### Introduction

*E. sakazakii* has been associated with outbreaks of a rare form of infant meningitis, necrotizing enterocolitis, bacteraemia and neonate deaths with a mortality rate of 40 to 80%<sup>(1,2)</sup>. Although *E. sakazakii* has been isolated from a diverse range of environments and foods, a dried infant formula has been a vehicle of transmission in the outbreaks and sporadic cases of *E. sakazakii*<sup>(3)</sup>. Powdered infant formulas are heat-treated during processing, but unlike liquid formula products they are treated with high temperatures for a sufficient time to make the final packaged product commercially sterile<sup>(4)</sup>. Although a heat treatment is reported as a general method for an inactivating or reducing *E. sakazakii*, a study on the thermal resistance of *E. sakazakii* in infant formula indicated that this species is one of the most thermotolerant bacteria in the *Enterobacteriaceae*<sup>(5)</sup>. The objectives of this study were to evaluate the inactivation effects of a gamma irradiation on *E. sakazakii* and the sensory properties of a dehydrated infant formula.

### Materials and Methods

Bacterial strain and Infant formula. The *E. sakazakii* strain KCTC 2949 was obtained from a Korean Collection for Type Cultures (KCTC, Daejeon, Korea). A can of commercial dehydrated infant formula for an age range of 3-5 months was purchased from a local supermarket. Background flora of *E. sakazakii* was tested and found to be negligible on the commercial product before inoculation.

Inoculation and Irradiation. The commercial dehydrated infant formula was inoculated with the *E. sakazakii* cocktail ( $10^9$  CFU/ml), was lyophilized at  $-70^\circ\text{C}$  and then blended using a sterile blender jar. To determine the amount of radiation necessary to eliminate 90% of the population ( $D_{10}$ -values), inoculated infant formula was irradiated with 0.0 (control), 1.0, 2.0, 3.0, or 4.0 kGy at the state of a powder 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, Korea.

Sensory analysis. The intensity sensory analysis of the irradiated infant formulas were performed at two different temperatures (room temperature and  $50^\circ\text{C}$ ) by an 11-member trained sensory panel. A 7-point scale was used for an analysis of the intensity of the color (1: white 7: brown) and flavor (milk flavor and off-flavor; 1: very weak 7: very strong) of the irradiated infant formula. In order to compare and calibrate, a control sample (non-irradiated) served as a reference at each session and was positioned in the middle of the scale except for the off-flavor (lowest score: 1). The samples were named with random numbers and their serving order was random for all the assessors.

## Results and Discussion

An irradiation effectively reduced the population of *E. sakazakii* in both the TSB and dehydrated infant formula (Fig. 1). A 2.5-log reduction of *E. sakazakii* was obtained at 1.0 kGy for the TSB. However, in the case of the dehydrated infant formula it was reduced a 1-log population by an irradiation at 1.0 kGy.

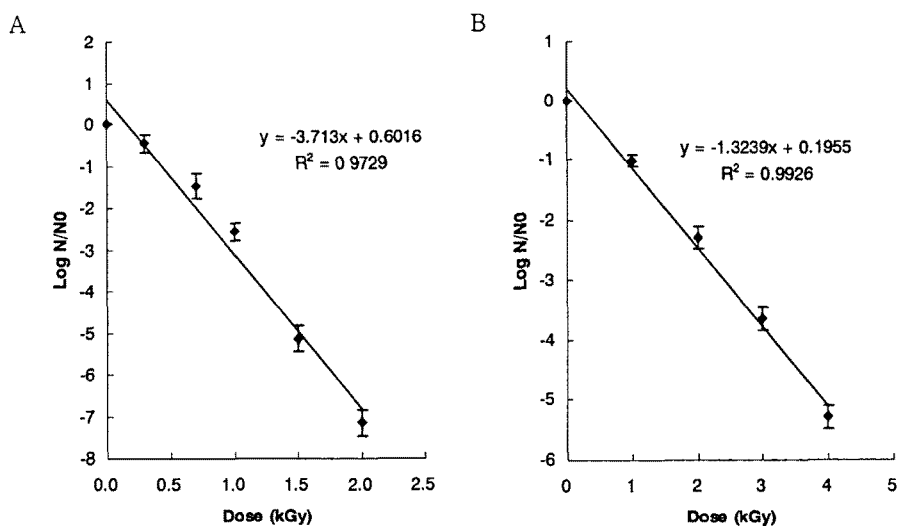


Fig. 1. Survival of *E. sakazakii* inoculated on broth (A) and dehydrated infant formula (B) after gamma-irradiation. The error bars represent the standard error.

In this study,  $D_{10}$ -value for *E. sakazakii* was  $0.27 \pm 0.05$  kGy and  $0.76 \pm 0.08$  kGy for TSB and dehydrated infant formula, respectively (Table 1). It may be due to a second effect (indirect action) by the products of a water radiolysis besides a direct action, a damage of the DNA by a gamma ray<sup>(6)</sup>.

Table 1. Radiation D-values (kGy) of *E. sakazakii* inoculated on broth and dehydrated infant formula

	$D_{10}$ -value
Tryptic soy broth	$0.27 \pm 0.05^a$
Dehydrated infant formula	$0.76 \pm 0.08$

<sup>a</sup>Numbers are D-values plus or minus the standard deviation of three different experiments with two measurement in each experiment.

Irradiation up to 5.0 kGy had no significant effect on the color and flavor of the dehydrated infant formula after a rehydration and a heating (Table 2).

Table 2. Sensory properties of irradiated infant formula after rehydration

Dose (kGy)	Color	Milk flavor	Off-flavor
<i>Room temperature<sup>a</sup></i>			
0	4.67±1.000 <sup>c N S</sup>	4.11±0.601 <sup>N S</sup>	1.89±0.928 <sup>N S</sup>
1	4.11±0.928	4.22±0.833	1.78±1.301
3	4.11±0.781	4.56±1.014	1.67±0.707
5	4.22±0.833	4.33±0.866	1.89±0.928
<i>Heated at 50°C<sup>b</sup></i>			
0	4.00±0.707 <sup>N S</sup>	4.00±0.866 <sup>N S</sup>	1.33±0.707 <sup>N S</sup>
1	4.33±1.118	3.78±0.972	1.56±1.013
3	4.78±0.600	4.11±0.928	1.89±1.054
5	4.11±0.601	4.11±1.054	2.11±0.782

<sup>N S</sup> No significant difference among the scores at the 5% level.

<sup>a</sup>Dehydrated infant formula was tested after rehydration by room temperature water.

<sup>b</sup>Dehydrated infant formula was tested after rehydration and heating at 50°C.

<sup>c</sup>Numbers are intensity score plus or minus the standard deviation.

In this study, a gamma irradiation completely eliminated *E. sakazakii* inoculated onto a dehydrated infant formula at a high level without effecting the sensory properties. However, considering the observed very low contamination levels (< 1 CFU/100 g) of *E. sakazakii* in the dehydrated infant formula<sup>(3)</sup>, an adequate inactivation level must be established to assure the safety and to minimize the losses of the nutritional properties.

## Reference

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