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Effects of Extended Dry Storage of Powdered Infant Milk Formula on Susceptibility of *Enterobacter sakazakii* to Hot Water and Ionizing Radiation

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ABSTRACT

Infant milk formula has been identified as a potential source of *Enterobacter sakazakii,* which has been implicated in neonatal meningitis and necrotizing enterocolitis. This study was undertaken to determine whether the length of *E. sakazakii* storage in powdered infant milk formula (PIMF) affected the ability of the pathogen to survive subsequent reconstitution of the powder with hot water or treatment with gamma radiation. Five *E. sakazakii* strains were mixed individually with PIMF and kept for up to 12 months at 25°C. After storage PIMF was reconstituted with water at 60 to 100°C or was exposed to ≤5 kGy of gamma radiation. Without any treatment secondary to drying, *E. sakazakii* counts decreased <1 log/g after 1 month but decreased about 4 log/g during storage for 8 to 12 months. Dry storage decreased thermal resistance but increased resistance of *E. sakazakii* to ionizing radiation in PIMF. Reconstitution of contaminated powder with water at 70°C after 1 month of dry storage reduced *E. sakazakii* viability slightly, >2 log/g, and after powder was stored for 12 months all *E. sakazakii* strains were eliminated. In contrast, desiccation substantially increased the resistance of *E. sakazakii* strains to ionizing radiation. Although the *D*-value for *E. sakazakii* IMF1 following overnight storage in PIMF was 0.98 kGy, >4 kGy was required to kill 1.5 log/g of the same strain that had survived 12 months in dry PIMF. Results suggested that low-dose irradiation will more effectively eliminate *E. sakazakii* from PIMF if the treatment is applied shortly after PIMF manufacture.

Enterobacter sakazakii is a gram-negative, facultatively anaerobic, rod-shaped bacterium. It is considered to be an opportunistic pathogen that has been regularly isolated from powdered infant milk formula (PIMF) *(4, 13, 16, 18, 27, 31)* even after extended periods (>2 years) of storage *(8).* The presence of *E. sakazakii* in PIMF has been linked to cases and outbreaks of *E. sakazakii* infections that are characterized by severe meningitis and necrotizing enterocolitis in infants *(1, 3, 9, 32).*

Apparently, the survival of this bacterium in PIMF (which has low water activity [a_w]) largely depends on its ability to counteract the stress of osmotic pressure changes during drying *(5).* Since endospore formation does not occur in this organism, *E. sakazakii* survives through other protective mechanisms *(5, 6).* At the onset of desiccation, *E. sakazakii* accumulates the solute trehalose inside cells, which stabilizes the shell-like layer of water around macromolecules and prevents structural damage *(5, 24).*

Thermal treatment of foods before consumption has long been used as a primary means to reduce the risks associated with foodborne pathogens *(21)* and has been identified as a practical means of reducing the risk from *E. sakazakii* in PIMF *(11).* The World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO) *(34)* recommended the use of hot water (70°C) to reconstitute PIMF before use at infant feeding to eliminate the pathogen.

Irradiation of food by ionizing energy without thermal treatment is considered an effective method of inactivating pathogens that may be present *(10, 14, 25).* Using ionizing radiation of up to 10 kGy can greatly reduce the number of spoilage organisms and eliminate pathogens without causing toxicological hazards *(33)* or compromising nutritional and sensory quality *(35).* The efficacy of reducing the viability of *E. sakazakii* in PIMF by ionizing radiation has been investigated *(23, 29),* but in these studies nondesiccated cells were used.

The aim of the current study was to determine the effect of lengthy storage in dry PIMF on the survival of *E. sakazakii* following reconstitution of the powder with hot water or following gamma radiation treatment of the stored, contaminated dry PIMF.

MATERIALS AND METHODS

PIMF samples. Cans containing 450 g of commercial PIMF (56.6% carbohydrate, 11.4% protein, and 25.4% fat) were obtained from a local processor. The formula was screened *(17)* and found free of *E. sakazakii.*

***E. sakazakii* strains.** One ATCC strain (strain 51329) and four food isolates originally isolated by Shaker et al. *(31)* from infant milk formulas (IMF1 and IMF2), infant food formula (IF1), and crushed wheat (CS1) at the Department of Nutrition and Food

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Technology, Jordan University of Science and Technology, Jordan, were used in the present study.

Culture preparation. Bacterial cultures were kept individually at −40°C in 5-ml vials containing brain heart infusion (BHI) (Oxoid, Basingstoke, UK) plus 20% (vol/vol) glycerol. For experiments, a loop of each *E. sakazakii* culture was grown individually at 37°C for 24 h in 15-ml tubes containing 10 ml of BHI. Cultures were used in experiments after five transfers. The individual cultures were centrifuged (Herolab, Wiesloch, Germany) at 3,000 × *g* for 20 min, and the pellets were resuspended in 1 ml of sterile potassium phosphate buffer (0.1 M, pH 6.8) to a concentration of approximately 10 log CFU/ml.

Preparation of desiccated *E. sakazakii*. *E. sakazakii* was mixed with PIMF as described by Osaili et al. *(29).* Briefly, 100 g of commercial PIMF was spread on the bottom of a sterile 50-cm-diameter stainless steel bowl and 0.5 ml of each culture was separately sprayed onto the powder by using a chromatography reagent sprayer at a nitrogen pressure of 0.138 bar. The treated powder was mixed thoroughly with a sterile spatula and passed through a sterile screen with 0.5-mm-diameter pores. The inoculated formulas were then stored at 25°C in 500-ml sterile, opaque screw-cap bottles (with 1.5 to 2 cm headspace) for 1 or 12 months for thermal inactivation studies or 1, 8, or 12 months for irradiation inactivation studies. Overnight stored, inoculated samples were considered controls. The quantity of *E. sakazakii* cells for inoculation of samples to be stored for 1 or 8 months was approximately 7 log CFU/g, while in samples to be stored for 12 months numbers were about 5 log CFU/g.

a_w measurement. The a_w of PIMF in 500-ml sterile, opaque screw-cap bottles was measured before and after inoculation with an a_w meter (Hygrolab, Rotronic Instrument Corp, Huntington, N.Y.).

Thermal inactivation of desiccated *E. sakazakii*. After the desired storage interval, 9 g of inoculated PIMF was transferred to sterile 150-ml-capacity plastic baby feeding bottles. The samples were reconstituted according to the manufacturer's instructions with 60 ml of sterile water at 60, 70, 80, 90, or 100°C. The bottles were closed with screw caps and gently agitated by hand at room temperature (25°C) for 10 min. During this period at 10-s intervals, temperature changes were monitored and recorded (TM99A, Cooper Instruments Corp., Middleton, Conn.) via a general-purpose thermocouple inserted through the nipple of a closed bottle. The experiments were repeated at least three times.

Irradiation inactivation of desiccated *E. sakazakii*. Following the storage intervals previously described, 10-g samples of the inoculated PIMF were placed in Seward stomacher bags (BA6040, Seward Ltd., Norfolk, UK) and the bags were sealed to be treated with gamma radiation. Samples were irradiated in a gamma research irradiator (Gamma-facility PX-γ-30, Issledovatelj, Techsnabexport, Moskivia) at the Jordan Atomic Energy Commission, Amman, Jordan. The spherical instrument had a sample capacity of 4,400 cm^3 and used Co^{60} as an irradiation source. The source activity was 2.04 kCi, with a dose rate of 1.21 kGy/h. The dose uniformity ratio, calculated as the ratio of the maximal dose and minimal dose absorbed in the irradiated material (D_{max}/D_{min}), was approximately 1. For dosimetry, 2-ml ethylene chlorobenzene dosimeters were used (Institute of Isotopes and Chemical Research Centre, Hungarian Academy of Sciences, Budapest), and the dosimeter response was measured with an oscillotitrator (OK-302/1, Radelkis, Budapest, Hungary). The dosimeters were calibrated against an international standard (RISØ, Roskilde, Denmark). The PIMF samples were exposed to radiation doses from 1 to 5 kGy, and the experiments were repeated at least three times.

Bacterial enumeration. Following reconstitution of PIMF with water in baby feeding bottles, *E. sakazakii* organisms were enumerated by spread plating duplicate aliquots (1 ml) of the samples and appropriate dilutions in 0.1% (wt/vol) peptone water (Becton Dickinson, Sparks, Md.) on tryptic soy agar (TSA; Oxoid). The plates were incubated aerobically at 37°C for 24 h. Samples (60 ml) in which *E. sakazakii* was not detected on TSA were enriched with 50 ml of BHI and incubated overnight at 37°C. Then, 0.1-ml aliquots of the enriched samples were spread on violet red bile salt glucose agar (VRBGA) (Oxoid) and incubated at 37°C for 24 h before examination for presumptive *E. sakazakii* colonies.

Irradiated and nonirradiated sample bags were opened aseptically, and their contents were transferred to sterile stomacher bags. Ten milliliters of 0.1% peptone water (Becton Dickinson) was added to each (10-g) sample. The diluted samples were massaged by hand for 2 min, and aliquots of the samples or dilutions were plated in duplicate on TSA.

D_{10}-value determination. Numbers of surviving *E. sakazakii* after radiation were entered into a Microsoft Excel spreadsheet (Microsoft, Redmond, Wash.) and analyzed for D_{10}-values (radiation dose required to inactivate 90% of the bacterial population) with Excel software. The logarithms of the number of surviving *E. sakazakii* cells in PIMF after radiation treatment were plotted against the radiation dose. The D_{10}-value was calculated from the linear regression model for the log of surviving bacterial cells and radiation dose. The D_{10}-value is the negative slope of the plot:

$$\log(N) = \log(N_0) - r/D$$

where N is the number of survivors (in CFU per gram) at radiation dose r (in kilograys) and N_0 is the number of organisms before irradiation.

Statistical analysis. The means of the D_{10}-values of *E. sakazakii* strains stored desiccated in PIMF for ≤12 months were compared with D_{10}-values of overnight-desiccated strains by Student's *t* test at a 0.05 significance level by using Statistical Analysis System (SAS, 1999) software. Significant differences among the D_{10}-values of the *E. sakazakii* strains in each storage period were calculated using the least-square means method at a 5% level of significance using SAS.

RESULTS

***E. sakazakii* survival in dry PIMF.** Numbers of viable *E. sakazakii* cells in PIMF decreased by <1 log CFU/g after 1 month and by about 4 log CFU/g after 12 months of storage at 25°C (data not shown). The a_w of uninoculated PIMF and PIMF measured 18 h after inoculation was 0.21 ± 0.01. After 1 month of storage the a_w values of test samples increased to 0.28 ± 0.01 and reached 0.31 ± 0.01 after 12 months of storage.

Effect of dry storage interval in PIMF on thermal sensitivity of *E. sakazakii*. *E. sakazakii* cells stored overnight in PIMF before reconstitution (undesiccated control) decreased by ≤1 log CFU/g after 10 min of rehydration with 60°C water but were reduced by 4.4 to 4.9 log CFU/g when 70°C water was used. Reconstitution of PIMF with water at 80°C was not sufficient to eliminate the 7 log CFU

TABLE 1. *Effects of the length of dry storage at 25°C on* E. sakazakii *survival in inoculated PIMF 10 min after powder reconstitution with water at 25 to 100°C*

Strain	No. of *E. sakazakii* survivors after indicated storage time at water temp (°C) of[a]:																	
	25			60			70			80			90			100		
	C	1 mo	12 mo	C	1 mo	12 mo	C	1 mo	12 mo	C	1 mo	12 mo	C	1 mo	12 mo	C	1 mo	12 mo
ATCC 51329	6.97 ± 0.23	5.59 ± 0.25	1.19 ± 0.12	6.00 ± 0.21	3.70 ± 0.18	0/3	2.54 ± 0.10	3/3	0/3	3/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
IMF1	7.21 ± 0.01	6.06 ± 0.89	1.54 ± 0.15	6.19 ± 0.04	4.30 ± 0.38	2/3	2.38 ± 0.15	3/3	0/3	3/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
IMF2	6.96 ± 0.16	6.08 ± 0.05	1.43 ± 0.05	6.00 ± 0.21	4.21 ± 0.61	0/3	2.05 ± 0.37	3/3	0/3	3/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
IF1	6.98 ± 0.01	6.05 ± 0.08	1.34 ± 0.15	6.28 ± 0.10	4.14 ± 0.45	0/3	2.48 ± 0.42	3/3	0/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
CS1	7.12 ± 0.23	6.10 ± 0.14	1.21 ± 0.23	6.35 ± 0.21	3.93 ± 0.72	0/3	2.54 ± 0.64	3/3	0/3	3/3	2/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

[a] Experimental controls and samples stored for 1 month in PIMF were initially inoculated with 7 log CFU *E. sakazakii* per g. Samples stored 12 months were initially inoculated with 5 log CFU of *E. sakazakii* per g. C, control, analyzed 18 h after inoculation. Values are means of three replications ± standard deviation (log CFU per gram) or are given as the ratio of samples positive for *E. sakazakii* by overnight enrichment in BHI and plating on VRBGA to number of samples analyzed.

TABLE 2. D_{10}*-values of* E. sakazakii *in PIMF stored for 1 and 8 months at 25°C*

E. sakazakii strain	D_{10}-values (kGy)[a]		
	Control samples	Samples stored for:	
		1 mo	8 mo
ATCC 51329	1.50 ± 0.03 B a	1.55 ± 0.18 B a	1.95 ± 0.24 A a
IMF1	0.98 ± 0.04 B b	0.96 ± 0.15 B b	1.28 ± 0.13 A b
IMF2	1.06 ± 0.15 A b	1.00 ± 0.12 A b	1.13 ± 0.25 A b
IF1	1.00 ± 0.05 AB b	0.95 ± 0.09 B b	1.09 ± 0.04 A b
CS1	0.94 ± 0.05 A b	0.98 ± 0.10 A b	1.08 ± 0.29 A b

[a] Values are means of three replications ± standard deviation. D_{10}-values were not calculated for 12-month storage samples because the initial levels present were very low. Within the same row, means with the same uppercase letter are not significantly different ($P > 0.05$). Within the same column, means with the same lowercase letter are not significantly different ($P > 0.05$).

E. sakazakii per g initially present in milk formula (Table 1).

The sensitivity of *E. sakazakii* to thermal challenge increased as the length of prior desiccated storage in PIMF increased (Table 1). When PIMF stored for 1 month was reconstituted with 60°C water, numbers of viable *E. sakazakii* were reduced by 1.7 to 1.9 log CFU/g compared with PIMF reconstituted with water at 25°C. However, after PIMF had been stored for 12 months (with the exception of strain IMF1) *E. sakazakii* cells were not recovered from similarly rehydrated samples (Table 1). Although rehydration at both 70 and 80°C failed to eliminate viable *E. sakazakii* from samples contaminated a month earlier, the bacterium was undetectable (<0.3 log CFU/ml) in similarly rehydrated samples that had been stored 12 months as dry powder. Regardless of inoculation level or PIMF storage period, *E. sakazakii* was not recoverable from samples reconstituted at 90 or 100°C.

Powder reconstituted at 100, 90, and 80°C remained above 70°C for 3.8, 2.7, and 0.8 min, respectively. When reconstituted at 70°C, the temperature reached 60°C within 0.8 min (data not shown).

Effect of gamma radiation (nonthermal inactivation). Although irradiation D_{10}-values of *E. sakazakii* ATCC 51329 were higher than those of the food isolates used in the present study ($P < 0.05$), the D_{10}-values of all *E. sakazakii* cultures tested after 1 month and most D_{10}-values at 8 months of storage in dry PIMF were not significantly different ($P > 0.05$) from control D_{10}-values determined in PIMF the day following powder inoculation (Table 2). The D_{10}-values of *E. sakazakii* ATCC 51329 and IMF1 stored for 8 months were significantly higher than those of the control and those stored for 1 month. Upon closer examination, the D_{10}-values of cells stored 8 months in PIMF before irradiation were higher than those of the controls by 7 to 31%. After ≥4 kGy treatment *E. sakazakii* organisms were not found in PIMF stored for 8 months when direct plating on TSA was used, but cells of strains ATCC 51329 and IMF1 were detectable following BHI en-

richment in two-thirds and one-third of samples, respectively, exposed to 5 kGy (Table 3). In contrast to the results from hot water reconstitution of contaminated dry powder, it was evident that extended dry storage in PIMF increased the resistance of *E. sakazakii* to ionizing radiation. For example, although a 2-log CFU/g reduction of the *E. sakazakii* food isolate strains in control samples would be achieved by 2 kGy (twice the D_{10}-value) (Table 2), the latter dose was insufficient to consistently eliminate 1.2 to 1.5 log of the same isolates in PIMF stored 12 months (Table 3).

While desiccation enhanced resistance to irradiation treatment, strains varied in terms of the extent of change in resistance development during extended dry storage. Even though *E. sakazakii* strain ATCC 51329 was most resistant at 1 month of storage, this strain and IMF2 were more sensitive than the others at 12 months of storage, when they were not detectable in PIMF subjected to 2-kGy treatments (Table 3). In contrast, strain IMF1 was recovered from PIMF samples irradiated at 4 kGy after 12 months of dry storage.

DISCUSSION

E. sakazakii is relatively resistant to osmotic and desiccation stress compared with other members of the *Enterobacteriaceae (5, 8).* Caubilla-Barron and Forsythe *(8)* found, using a mixture of strains, that *E. sakazakii* survived over 2 years of storage in infant formula at room temperature. In addition, *E. sakazakii* was found to be resistant to desiccation in powdered infant formulas over a wide range of a_w (0.25 to 0.86) and temperatures (4 to 30°C) *(15).*

The ability of *E. sakazakii* strains to survive in PIMF observed in the present study is consistent with the results of previously published work. Caubilla-Barron and Forsythe *(8)* reported that the viability of *E. sakazakii* decreased by 3.3 and 4.5 log CFU/g after 6 and 30 months of storage, respectively, at room temperature. Similarly, Edelson-Mammel et al. *(12)* found, with a single strain, that *E. sakazakii* levels (6 log CFU/g) in PIMF (a_w, 0.14 to 0.27) decreased about 2.5 and 3.5 log during 8 and 24 months of storage at 21°C, respectively, which is lower than the reduction observed in the present study. The slight differences between the results of the former and present studies may be explained by the differences in a_w levels and storage temperature. Gurtler and Beuchat *(15)* reported that the death rate of *E. sakazakii* in powdered infant formula increased as a_w and storage temperature increased. These authors were able to detect *E. sakazakii* by enrichment in 6 of 6, 4 of 6, and 1 of 6 formulas stored for 12 months at 4, 21, and 30°C, respectively. Breeuwer et al. *(5)* found that *E. sakazakii* dried in air and incubated for 46 days at 25°C decreased 1 to 1.5 log units from the initial level of 9 log CFU/ml. The resistance of *E. sakazakii* to desiccation was thought by Breeuwer et al. *(5, 6)* to be due to the accumulation of trehalose inside the cells and to the expression of heat shock and stringent response regulons. Trehalose seems to act by dissolving in the shell of water around macromolecules, thus preventing damage to the cells *(24).*

The increase in a_w of PIMF observed during storage

TABLE 3. *Effects of the length of dry storage at 25°C on survival of each of five* E. sakazakii *strains in inoculated PIMF subsequently treated with ≤5 kGy ionizing radiation*

Strain	No. of *E. sakazakii* survivors, after indicated no. of months of storage, in PIMF treated with radiation dose of[a]:											
	0 kGy		1 kGy		2 kGy		3 kGy		4 kGy		5 kGy	
	8 mo	12 mo	8 mo	12 mo	8 mo	12 mo	8 mo	12 mo	8 mo	12 mo	8 mo	12 mo
ATCC 51329	2.54 ± 0.27	1.19 ± 0.12	1.56 ± 0.15	3/3	1.01 ± 0.19	0/3	3/3	0/3	3/3	0/3	2/3	ND
IMF1	2.57 ± 0.09	1.54 ± 0.15	1.62 ± 0.32	3/3	1.00 ± 0.11	6/6	3/3	2/6	3/3	2/6	1/3	ND
IMF2	2.85 ± 0.05	1.43 ± 0.05	1.78 ± 0.11	3/3	1.03 ± 0.32	0/3	3/3	0/3	0/3	0/3	0/3	ND
IF1	3.29 ± 0.03	1.34 ± 0.15	2.17 ± 0.15	3/3	1.45 ± 0.09	4/6	3/3	0/3	3/3	0/3	0/3	ND
CS1	2.96 ± 0.51	1.21 ± 0.23	1.90 ± 0.44	3/3	1.01 ± 0.40	2/6	3/3	0/3	0/3	0/3	0/3	ND

[a] Samples stored 8 or 12 months were inoculated initially with 7 log or 5 log CFU of *E. sakazakii* per g, respectively. Values are means of three replications ± standard deviation (log CFU per gram) or are given as the ratio of number of samples positive for *E. sakazakii* by enrichment to number of samples analyzed. ND, not determined.

in the present study was probably due to the water vapor gained during opening of the bottles for sampling *(12).* Thermal inactivation of *E. sakazakii* in infant milk formula has been studied and was afforded special attention by the WHO/FAO *(34).* Heat treatment remains the principal means of eliminating this foodborne pathogen from PIMF during its reconstitution. Nazarowec-White and Farber *(28)* found that the thermal *D*-value for *E. sakazakii* in reconstituted PIMF at 58°C was 4.2 min. Other researchers reported lower *D*-values at the same temperature (0.27 to 2.6 min) for *E. sakazakii (5, 19).* Reconstitution of PIMF with water at 70°C reported here yielded exposures of >58°C for at least 2.5 min. At 60°C treatment, organisms were exposed to 58°C for about 10 s. The present results from inactivation of briefly (overnight) desiccated *E. sakazakii* in control PIMF treatments by reconstitution with hot water are similar to those of Edelson-Mammel and Buchanan *(11)* who found that addition of water at 60 to 70°C decreased the levels of undesiccated *E. sakazakii* by 1 to 4 log. In most of the research conducted on thermal inactivation of *E. sakazakii,* strains that were prepared under optimal laboratory conditions were used. However, studying the thermotolerant properties of desiccated *E. sakazakii* in PIMF better reflects the actual physiological state of the microbe as it exists in PIMF. The decrease in thermal resistance of desiccated *E. sakazakii* in the present study could be explained by the sequential exposure of the organisms to two different stresses. Exposure of the test organisms here to desiccation stress was combined with starvation stress, and for maintenance of viability, energy-consuming production of protective stress shock proteins may have been required, causing the microorganisms to be metabolically exhausted *(2)* and less heat resistant. Further study is needed to explain this phenomenon.

Irradiation D_{10}-values of 0.95 to 1.95 kGy obtained in the present study for *E. sakazakii* either briefly desiccated or desiccated for ≤8 months in PIMF and those previously reported by Osaili et al. *(29)* and Lee et al. *(23)* for undesiccated *E. sakazakii* in infant milk formula show that *E. sakazakii* can be controlled by low radiation doses. Osaili et al. *(30)* found that acid, alkaline, chlorine, and ethanol stresses decreased the D_{10}-values of the same *E. sakazakii* strains used in the present study. In contrast, Buchanan et al. *(7)* found that acid stress cross-protected *Escherichia coli* in a broth system against radiation by expression of *rpoS*-regulated genes in the cells, whereas Kim and Thayer *(22)* found that heating *Salmonella* Typhimurium for 2 min at 65°C before irradiation treatment decreased the D_{10}-values by 34%.

Based on *D*-values observed in milk powder stored at 25°C for up to 8 months, 2 log CFU of *E. sakazakii* per g should have been eliminated by 2-kGy treatment. However, when irradiation was done at higher energy levels, survival of *E. sakazakii* was greater than predicted. Viable cells of two strains were isolated following enrichment of PIMF treated with 5 kGy after 8 months of dry storage. Thus, extended drying enabled development of substantial resistance to irradiation by some strains.

The increase in resistance of desiccated *E. sakazakii* toward ionizing radiation may be related to a loss of free water inside the cells during desiccation, which may have decreased the indirect damage of ionizing radiation through the reduction of free-radical formation in the cells *(14, 20).* Some protection by expression of genes during storage which generated shock response proteins is also possible.

In summary, *E. sakazakii* has significant ability to survive desiccation in PIMF. Using water at ≥60°C to reconstitute PIMF would be effective in eliminating the highest reported levels of *E. sakazakii* in PIMF, which were 66 CFU/100 g *(26).* Low doses of ionizing radiation, although not currently approved for this purpose, could be used to eliminate possible contamination of PIMF by *E. sakazakii* during processing but should be used without extended storage of powder before irradiation. The use of hot water and gamma radiation may reduce the risk of *E. sakazakii* associated with PIMF; however, these treatments should not be substitutes for good manufacturing and hygienic practices.

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