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Synergistic Bactericidal Effect of Simultaneous Near-Infrared Radiant Heating and UV Radiation against *Cronobacter sakazakii* in Powdered Infant Formula

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The aim of this study was to investigate the synergistic bactericidal effects of the simultaneous application of near-infrared (NIR) heating and UV irradiation against *Cronobacter sakazakii* in powdered infant formula and to determine the effect on quality by measuring color changes and performing sensory evaluation. A cocktail of *C. sakazakii* strains was inoculated into powdered infant formula, followed by NIR, UV, and combined NIR-UV treatments. The sum of NIR and UV inactivation was lower than that obtained by the simultaneous application of both technologies due to their synergism. Simultaneous NIR-UV combined treatment for 7 min achieved a 2.79-log-unit CFU reduction of *C. sakazakii*. The underlying inactivation mechanisms of the combined NIR-UV treatment were evaluated by the propidium iodide (PI) uptake test, and we confirmed that disruption of the bacterial cell membrane was the main factor contributing to the synergistic lethal effect. The color values and sensory characteristics of simultaneously NIR-UV-treated infant formula powder were not significantly ($P > 0.05$) different from those of the control. The results of this study suggest that a NIR-UV decontaminating system can be applied as an alternative to other interventions in powdered weaning foods.

Cronobacter sakazakii was first identified as yellow-pigmented *Enterobacter cloacae* in 1980 and then was reclassified into several genomospecies (1, 2). While *C. sakazakii* has been a causal agent of diseases among all age groups, the great majority of cases are seen in infants less than 2 months old. *C. sakazakii* has been associated with food-borne outbreaks of a rare form of infant meningitis, necrotizing enterocolitis, bacteremia, and neonate deaths, with a fatality rate of 40% to 80% (3). Although *C. sakazakii* has been isolated from a diverse range of environments and foods, dried infant formula, which is a main source of nutrition for neonates and infants, has been recognized as the major vehicle of transmission in *C. sakazakii* infections (3, 4, 5). An international survey of powdered infant formulas from 36 countries found that approximately 14% of the 141 canned products examined had detectable levels of *C. sakazakii* (6). A smaller survey by Nazarowec-White and Farber (7) involved 5 factories (each supplying 24 samples) and reported that the prevalence of the bacterium ranged between 0% and 12% of the samples per factory. Iversen and Forsythe (8) also found that 2.4% of powdered infant formula products (in 82 brands) were positive for *C. sakazakii*.

Most studies involving inactivation of *C. sakazakii* in infant formula have focused on the reconstituted/rehydrated state, not the end product in powdered form (9, 10, 11, 12, 13). Drying is an extreme form of osmotic stress and is important for the survival and persistence of *C. sakazakii* in the powder bed. *C. sakazakii* has been known to be more resilient in low-water-activity environments (14, 15). Due to its ability to survive in dry food matrices, as in powdered infant formula, controlling *C. sakazakii* in the final dehydrated product is of great concern to the dairy industry.

Recently, some authors have proposed several decontamination methods in an effort to reduce levels of *C. sakazakii* in powdered infant formula, including gamma radiation (16, 17), electron beam irradiation (18), supercritical carbon dioxide and heat treatment (19), and gaseous ozone treatment (20). However, all of these methods have limitations for industrial application because of high capital costs for installation and treatment. Therefore, it is necessary to develop a more efficient method for inactivation of *C. sakazakii* that can be applied to the final dehydrated product. This new intervention should be cost-effective, and its impact on product quality should enable its application in postpasteurization processing of powdered infant formula.

Infrared (IR) energy is a form of electromagnetic energy that, if absorbed, can cause heating on the surfaces of objects and is distinguished as near IR (NIR) (0.76 to 2 μm), medium IR (MIR) (2 to 4 μm), and far IR (FIR) (4 to 1,000 μm). IR heating has advantages over convection and conduction heating, as it heats the product directly without being influenced by air around the powdered infant formula and is a fast and effective thermal process (21). This rapid surface heating by IR can help improve the sealing of moisture, flavor, and aroma compounds, leading to products with better sensory characteristics (22). On the other hand, 253.7-nm UV irradiation (UV-C) holds considerable promise in food processing as an emerging nonthermal method for microbial inactivation on food surfaces. UV-C radiation has been recommended for use in combination with other antimicrobial techniques, since cumulative damage based on microbial DNA appears to be effective in inducing the synergistic inactivation (23). In our previous study, the simultaneous combination of NIR heating and UV-C irradiation was found to be suitable for reducing

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Salmonella enterica serovar Typhimurium and *Escherichia coli* O157:H7 in red pepper powder without affecting product quality (24). However, the feasibility of combined NIR-UV treatment for controlling *C. sakazakii* in dehydrated infant formula has not yet been evaluated, which is one of the purposes of the current study.

In this study, the efficacy of simultaneous near-infrared heating and UV-C irradiation for reducing populations of *C. sakazakii* in powdered infant formula was investigated. The mechanism of inactivation was evaluated by propidium iodide (PI) uptake values. Also, the effect of the combination treatment on the quality of powdered infant formula was determined by measuring the color change and by sensory evaluation.

MATERIALS AND METHODS

Bacterial strains. Three strains of *C. sakazakii* (ATCC 12868, ATCC 29544, and ATCC 51329) were obtained from the Bacterial Culture Collection of Seoul National University (Seoul, South Korea) and were used in the experiments. Stock cultures were kept frozen at −80°C in 0.7 ml of tryptic soy broth (TSB) (Difco Becton, Dickinson, Sparks, MD, USA) and 0.3 ml of 50% (vol/vol) glycerol. Working cultures were streaked onto tryptic soy agar (TSA) (Difco), incubated at 37°C for 24 h, and stored at 4°C.

Preparation of pathogen inocula. All strains of *C. sakazakii* were cultured individually in 5 ml of TSB at 37°C for 24 h, harvested by centrifugation (4,000 × *g* for 20 min at 4°C), and washed three times with buffered peptone water (BPW) (Difco). The final pellets were resuspended in BPW, corresponding to approximately 10^6 to 10^7 CFU/ml. Subsequently, the suspended pellets of each strain of the *Cronobacter* spp. were combined to produce mixed culture cocktails (three strains in total). These cell suspensions, consisting of a final concentration of ca. 10^7 CFU/ml, were used in the inactivation study. To analyze the mechanism of inactivation, a final pellet of *C. sakazakii* was resuspended in 5 ml of phosphate-buffered saline (PBS) (0.1 M) and inoculated into a sterile glass petri dish (16 mm [height] by 90 mm [inside diameter]).

Sample preparation and inoculation. Commercial powdered infant formula (Namyang Co., Gongju, South Korea) was purchased at a local grocery store (Seoul, South Korea). For inoculation, 6 ml of culture cocktail was added dropwise to 250-g samples inside sterile high-density polyethylene (HDPE) bags (300 mm by 450 mm). The inoculated samples were thoroughly mixed by hand massaging for 10 min to produce a homogeneous dispersal of inoculum throughout the powered infant formula and dried for 2 h inside a biosafety hood (22 ± 2°C) with the fan running until the water activity (a_w) of the sample equaled that of an uninoculated sample (ca. 0.42). The water activities of uninoculated and inoculated samples were measured using the AquaLab model 4TE water activity meter (Decagon Devices, Pullman, WA, USA). The final cell concentration was 10^5 to 10^6 CFU/25 g. The inoculated infant formula powder samples were then immediately used in each experimental trial.

Near-infrared heating and UV irradiation. NIR and UV-C treatments were carried out in a previously described apparatus (24). A stainless steel chamber (concave-upwards base, 380 by 205 by 158 mm) with a rotational mixer was used for combined NIR and UV-C treatment (Fig. 1). A UV germicidal lamp (G10T5/4P; 357 mm; Sankyo, Japan) with a nominal output power of 16 W (radiation intensity, 2.62 mW/cm^2 at the sample location) was used as a UV-C-emitting source. A quartz halogen infrared heating lamp (NS-104; 350 mm; NSTECH, South Korea) with a maximum power of 500 W (radiation intensity, 141.75 μW/cm^2/nm at the sample location) at a 230-V input was used as a NIR-emitting source. The maximum wavelength (λ_m) generated from the infrared heater used in this study was about 1,300 nm, which is in the near-infrared wavelength range. Since both lamps radiate in all directions, two aluminum reflectors were installed behind the emitters to redirect the radiation waves and enhance the efficiency of NIR and UV radiation (Fig. 1). After the outputs of the NIR and UV lamps had been stabilized (following 2 min of run time), inoculated samples (250 g) were placed in the treatment chamber for the subsequent inactivation experiments (NIR radiant heating, UV-C irradiation, and simultaneous application of both technologies). All treatments were accompanied by stirring (23 rpm) by means of a rotational mixer in the chamber (Fig. 1). For the inactivation mechanism study, 5-ml cell suspensions kept in glass petri dishes were treated with NIR, UV, and NIR-UV for 3 min under identical conditions. The volume of the cell suspension (5 ml) and the treatment time (3 min) were selected on the basis of the temperatures of powdered infant formula observed during NIR treatment.

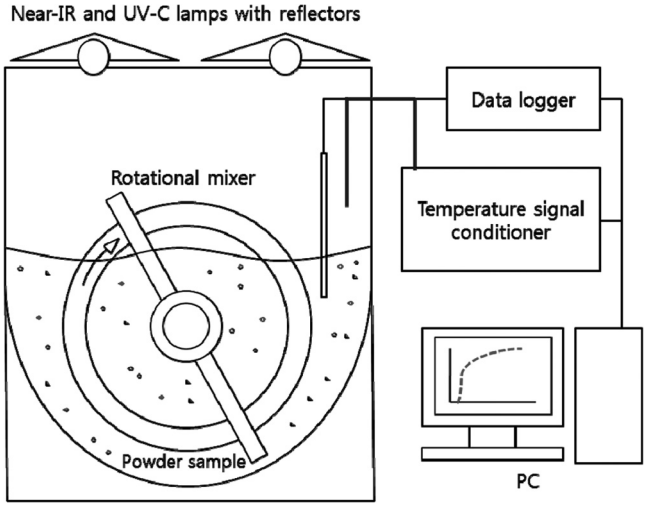

FIG 1 Schematic diagram of the combined NIR-UV treatment system used in this study.

Temperature measurement. A fiber optic temperature sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada) was used to measure real-time temperatures in the treatment chamber during NIR-UV combined treatment. The sensor was placed directly on the inner wall surface of the chamber, and the temperature was manually recorded every 10 s. Additionally, in order to measure the core temperature of treated samples precisely, a K-type thermocouple and a data logger (34790A; Agilent Technologies, Palo Alto, CA) were used (Fig. 1). The thermocouple probe was directly inserted into the infant formula powder bed, and temperatures were recorded at selected treatment times. All experiments were replicated three times.

Bacterial enumeration. At selected time intervals, 25-g treated samples were removed and immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of BPW (detection limit, 10 CFU/g) and homogenized for 2 min with a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1-ml aliquots of sample were 10-fold serially diluted in 9 ml blanks of BPW, and 0.1 ml of sample or diluent was spread-plated onto selective medium, chromogenic *Enterobacter sakazakii* agar (ESA) (Brilliance, DFI formulation; Oxoid), for the enumeration of *C. sakazakii* cells. The agar plates were incubated at 37°C for 24 h, and then the cells were enumerated by counting blue-green colonies.

Enumeration of injured cells. The liquid repair method was used to enumerate injured cells of *C. sakazakii*. One-milliliter aliquots of treated sample were 10-fold serially diluted in 9 ml of *Enterobacteriaceae* enrichment broth (Mossel formula; LAB, United Kingdom), and the diluted medium was incubated at 37°C for 2 h to allow injured cells to be resuscitated. After the recovery step, 0.1 ml of diluent was spread-plated onto chromogenic selective medium. All agar plates were incubated for 22 h at 37°C, and the typical blue-green colonies were counted. It has been reported that the optimal temperature range for growth of *Cronobacter*

strains is 37°C (25). Injured cells are easily resuscitated on nonselective broth or liquid medium in less than 2 h, and the liquid medium resuscitation method is simpler and faster than solid agar repair methods, such as the overlay (OV) method (26). By performing preliminary experiments, we confirmed that the 2-h incubation period in liquid broth did not cause multiplication of uninjured cells in control samples and that the recovery level of injured *C. sakazakii* cells in liquid broth was not significantly different from that in the agar OV method (TSA-ESA).

Assessment of propidium iodide uptake. Cell membrane damage induced by each treatment was quantitatively assessed by using the fluorescent dye PI (Sigma-Aldrich). Untreated, UV-treated, NIR-treated, and NIR-UV-treated *C. sakazakii* cells were centrifuged (10,000 × *g* for 10 min), and the cell pellets were resuspended and diluted in PBS to an optical density at 680 nm (OD_{680}) of approximately 0.2 and then mixed with PI solution to a final concentration of 2.9 μM. After incubation for 10 min, the samples were centrifuged at 10,000 × *g* for 10 min and washed twice in PBS to remove excess dye. The final cell pellets were resuspended in PBS, and fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 493 nm and an emission wavelength of 630 nm. Fluorescence values for each sample were normalized with the OD_{680} of the cell suspensions, and data obtained for untreated cells were subtracted from those for treated cells.

Color measurement and sensory evaluation. To determine the effect of NIR-UV treatment on the color of infant formula powder, a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan) was used to measure the color changes of treated samples. The color attributes were quantified by the values of L*, a*, and b* and measured at random locations on powdered infant formula. L*, a*, and b* values indicate color lightness, redness, and yellowness of the sample, respectively. All measurements were taken in triplicate.

Sensory evaluation was performed to determine how specific attributes (color, odor, and overall acceptability) varied over NIR-UV-treated powder samples (1-, 3-, 5-, and 7-min exposures) compared to those of a nontreated control. In all sensory tests, the panelists consisted of 12 members (5 men and 7 women; age range, 25 to 30 years) from the Department of Food and Animal Biotechnology, Seoul National University, Seoul, South Korea, and scores were obtained by rating the sensory attributes using the following 7-point hedonic scales: color intensity (7, brown; 1, white) and odor and overall acceptability (7, very good; 6, good; 5, below good/above fair; 4, fair; 3, below fair/above poor; 2, poor; 1, very poor). Powder samples, labeled with three-digit random numbers, were placed on white paper plates and presented after being cooled to room temperature. The presentation order was randomized, and the panelists were asked to use water to clean their palates between samples.

Statistical analysis. All experiments were repeated three times with duplicate samples. Triplicate data were analyzed by analysis of variance (ANOVA) with Duncan's multiple-range test of a statistical analysis system (SAS Institute, Cary, NC, USA). A P value of <0.05 was used to indicate significant differences.

RESULTS

Synergistic bactericidal effect of simultaneous NIR-UV treatment. The reduction in numbers of *C. sakazakii* cells in infant formula powder during NIR radiant heating, UV-C irradiation, and simultaneous application of both technologies is presented in Table 1. Reductions of 1.55, 2.11, and 2.79 log units were observed in *C. sakazakii* after simultaneous NIR-UV combined treatment for 5, 6, and 7 min, respectively. The sums of results for NIR and UV inactivation were lower than values obtained by the simultaneous application of both technologies. In other words, synergistic effects were observed for all treatment times against *C. sakazakii* (Table 1). However, statistically significant ($P < 0.05$) differences between the sums of NIR and UV inactivation and values for inactivation achieved with combination treatment were observed only after treatment times of 6 min or more. Log reductions resulting from the synergistic effect after 6 min of treatment, calculated by subtracting the sums of NIR and UV reductions from the values obtained during simultaneous NIR-UV treatment, were 0.73 and 0.95 log units at 6 and 7 min of treatment, respectively.

TABLE 1 Reductions in numbers of viable *C. sakazakii* cells in powdered infant formula treated with UV-C irradiation, NIR radiant heating, and simultaneous application of both technologies

Treatment time (min)	Log reduction [$\log_{10}(N_0/N)$] by treatment type and selective medium[a]			
			NIR-UV	
	UV (SA)	NIR (SA)	SA	SAR
0	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 Aa	0.00 ± 0.00 Aa
1	0.02 ± 0.02 AB	0.07 ± 0.09 A	0.10 ± 0.10 ABa	0.22 ± 0.20 Aa
2	0.01 ± 0.01 AB	0.14 ± 0.13 AB	0.33 ± 0.11 BCa	0.29 ± 0.22 Aa
3	0.01 ± 0.01 AB	0.39 ± 0.14 B	0.47 ± 0.09 Ca	0.62 ± 0.19 Ba
4	0.04 ± 0.06 AB	0.67 ± 0.16 C	0.81 ± 0.07 Da	0.93 ± 0.18 Ca
5	0.05 ± 0.03 AB	1.20 ± 0.18 D	1.55 ± 0.12 Ea	1.32 ± 0.25 Da
6	0.06 ± 0.04 AB	1.32 ± 0.29 D	2.11 ± 0.19 Fa	1.82 ± 0.17 Ea
7	0.07 ± 0.06 B	1.77 ± 0.01 E	2.79 ± 0.22 Ga	2.43 ± 0.08 Fa

[a] The values are means ± standard deviations from three replications. Values in the same column followed by the same uppercase letter are not significantly different ($P > 0.05$). Values in the same row followed by the same lowercase letter are not significantly different ($P > 0.05$). SA, plating directly on selective agar; SAR, plating on selective agar preceded by a resuscitation step. N_0, initial population; N, population after treatment.

Recovery of NIR-UV-injured cells. Levels of sublethally injured *C. sakazakii* cells in powdered infant formula following simultaneous NIR-UV treatment are shown in Table 1. Determining the difference between inactivation of samples subjected to the injured-cell resuscitation method and that of samples plated directly on selective media revealed the presence of 0.23, 0.29, and 0.36 log units of injured *C. sakazakii* cells after 5-, 6-, and 7-min treatments, respectively. Slightly smaller reductions of *C. sakazakii* numbers were observed at the final stages of the treatment (5, 6, and 7 min) by the procedure involving the resuscitation step (liquid broth recovery method) than by direct plating on selective agar (ESA). However, no statistically significant ($P > 0.05$) differences between levels of surviving cells, including sublethally injured *C. sakazakii* cells, were observed during the entire treatment time (Table 1).

Average temperature-time histories of powdered infant formula. Figure 2 shows average temperatures of the treatment chamber and infant formula powder core during simultaneous NIR and UV treatment. About 6 and 3°C temperature differences were observed between inside the chamber and the infant formula powder core at 1 and 2 min of treatment, respectively. This temperature disparity continued to decrease with treatment time, and from 3 min of treatment, the temperatures of the sample core and treatment chamber reached equilibrium (ca. 50°C). At maximum treatment time (7 min), the temperatures increased to ca. 73°C (Fig. 2). Additionally, the heating rate of NIR treatment alone was not different from that of simultaneous NIR-UV treatment of infant formula powder (data not shown).

Determination of cell membrane damage by PI uptake. As a quantitative analysis of membrane damage, NIR-, UV-, and NIR-UV-treated cells were stained with the fluorescent dye PI, which passes through only damaged cell membranes. The PI uptake values of *C. sakazakii* after each treatment are shown in Table 2. The

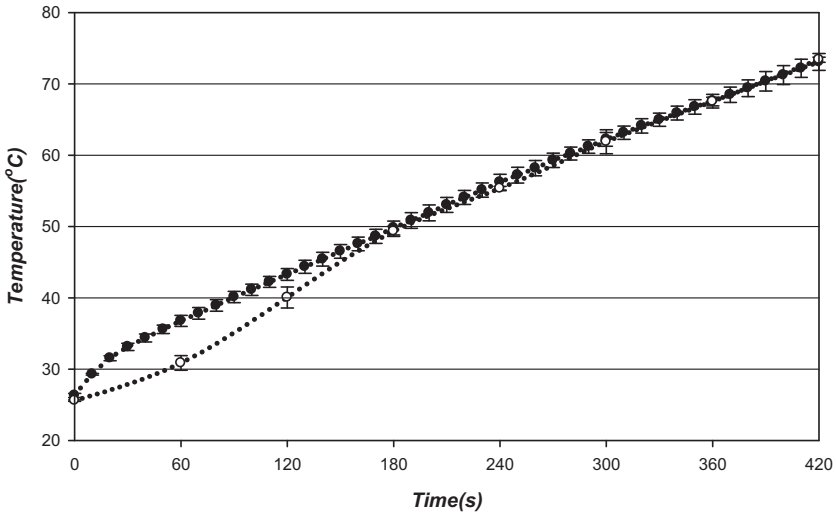

FIG 2 Average temperature-time histories for treatment chamber and infant formula powder cores during simultaneous NIR heating and UV-C irradiation. The error bars indicate standard deviations calculated from triplicates. ○, infant formula powder core; ●, inside treatment chamber.

degree of PI uptake in NIR- and NIR-UV-treated cells was much higher than that in UV-treated cells, indicating there was no significant damage to cellular membranes of *C. sakazakii* following UV treatment. Cells subjected to simultaneous NIR-UV treatment showed significantly ($P < 0.05$) higher PI uptake values than did cells subjected to the other treatments (Table 2).

Effect of simultaneous NIR-UV treatment on product quality. The color values of infant formula powder after combined NIR-UV treatment are summarized in Table 3. The L*, a*, and b* values of NIR-UV-treated (7 min) powdered infant formula were not significantly ($P > 0.05$) different from those of nontreated samples. Although the b* value (yellowness) slightly decreased with prolonged treatment time, statistically significant differences were not detected during the entire treatment interval (Table 3). Table 4 shows the sensory attributes of powdered infant formula following NIR-UV treatment. There were no significant ($P > 0.05$) differences among all tested samples scored by the hedonic scale for color, odor, and overall acceptability, indicating that simultaneous application of NIR and UV treatment for 7 min does not alter the sensory quality of the infant formula powder product significantly.

DISCUSSION

The primary means of pasteurization in the manufacture of powdered foods is heating. However, *C. sakazakii* has been known to be more thermotolerant than any other member of the *Enterobacteriaceae* (7), although thermal resistance among *C. sakazakii* strains varied as much as 20-fold (10). Moreover, thermal treatments cannot be efficiently applied to powdered matrices because of their low thermal conductivity. Kim et al. (19) reported that heat treatment at 63, 68, and 73°C for 30 min did not reduce populations of *C. sakazakii* in dehydrated infant formula. In our study, NIR treatment alone was also insufficient to inactivate *C. sakazakii* by the required amount (ca. 3-log-unit reduction) in infant formula powder (Table 1). To obtain a 3-log-unit reduction, treatment for 9.6 min was needed for *C. sakazakii* based on the calculated parameters of the Weibull model ($R^2 = 0.95$). However, yellowing and darkening were visually observed on infant formula powder treated with NIR for a slightly excessive time (over 9 min).

Inactivation of *C. sakazakii* utilizing hurdle combinations has been performed in infant formula by several researchers. Pina-Pérez et al. (27) obtained 2.2-log-unit reductions in numbers of *C. sakazakii* cells in reconstituted infant formula by the sequential combination of pulsed-electrical-field treatment and refrigerated storage (8°C). They also reported that microwave heating followed by refrigerated storage (5°C) resulted in the progressive

TABLE 2 Levels of membrane damage of UV-, NIR-, and simultaneously NIR-UV-treated cells obtained from the PI uptake test

Treatment type	*C. sakazakii* PI uptake value[a]
Untreated control	0 ± 0 A
UV	2.18 ± 0.13 B
NIR	13.21 ± 2.21 C
NIR-UV	28.76 ± 0.47 D

[a] The values are means of three replications ± standard deviations. Values followed by the same letters are not significantly different ($P > 0.05$). The data were normalized by subtracting fluorescence values obtained from untreated cells and against the OD_{680} as follows: PI value = (fluorescence value after treatment − fluorescence value of untreated cells)/OD_{680}.

TABLE 3 Color values of simultaneously NIR-UV-treated infant formula powder

Treatment time (min)	Color value for parameter[a]		
	L*	a*	b*
0	94.13 ± 0.66 A	−4.42 ± 0.08 A	21.80 ± 0.35 A
1	94.27 ± 0.75 A	−4.45 ± 0.31 A	21.98 ± 0.27 A
2	94.23 ± 0.66 A	−4.31 ± 0.03 A	21.90 ± 0.26 A
3	94.22 ± 0.64 A	−4.33 ± 0.11 A	21.78 ± 0.19 A
4	94.30 ± 0.64 A	−4.37 ± 0.09 A	21.66 ± 0.34 A
5	94.17 ± 0.59 A	−4.17 ± 0.13 A	21.44 ± 0.38 A
6	94.25 ± 0.69 A	−4.11 ± 0.16 A	21.26 ± 0.63 A
7	94.29 ± 0.76 A	−4.21 ± 0.38 A	21.17 ± 0.67 A

[a] The values are the means of three replications ± standard deviations. Values followed by the same letters within each column are not significantly different ($P > 0.05$).

TABLE 4 Sensory attributes of powdered infant formula following simultaneous NIR-UV treatment (at day 0)

Treatment time (min)	Sensory attribute score[a]		
	Color	Odor	Overall
0	4.0 ± 0.76 A	5.4 ± 0.74 A	5.1 ± 0.83 A
1	4.0 ± 0.93 A	5.1 ± 0.99 A	5.4 ± 0.92 A
3	3.9 ± 0.83 A	5.0 ± 0.93 A	5.4 ± 0.52 A
5	4.1 ± 0.83 A	5.1 ± 1.25 A	5.1 ± 1.13 A
7	3.8 ± 0.89 A	5.4 ± 0.52 A	5.1 ± 0.64 A

[a] Results are from panelist scorecard analysis on a 7-point hedonic scale. For color, 7, brown; 1, white. For odor and overall acceptability, 7, very good; 1, very poor. $n = 12$. The values are means. Values followed by the same letters within each column did not differ significantly ($P > 0.05$).

death of *C. sakazakii* in the rehydrated form (28). UV radiation-exposed infant formula powder reconstituted in 55, 60, and 65°C hot water demonstrated either additive or synergistic effects for inactivation of *C. sakazakii* (29). The implementation of multiple-hurdle technology in food matrices is becoming more attractive in terms of product quality because individual treatments, especially heating, can be used at lower intensity. The use of excessive heat for inactivation of bacteria results in denaturation of whey proteins, as well as changes in physical, mechanical, and optical properties of powdered infant formula. In the present study, simultaneous NIR and UV treatments resulted in greater reductions in cell numbers of *Cronobacter* spp. than did either treatment alone as a result of synergism (Table 1). Additionally, due to the shorter NIR processing time, combined NIR-UV treatments did not change the color values or sensory attributes of infant formula powder significantly ($P > 0.05$) (Tables 3 and 4).

Although UV radiation is well established for sanitization of air, water, and liquid food and for food surface decontamination, UV radiation as an intervention for powdered foods is still under investigation. Liu at al. (29) treated powdered infant formula with UV-C radiation to inactivate *C. sakazakii*. Treatment with UV radiation for 25 min (60.7 kJ/m^2) reduced *C. sakazakii*'s presence by 1.38 log CFU/g; however, complete inactivation was not achieved because UV radiation did not completely penetrate the dry infant formula. To overcome these limitations of the penetration capability of UV radiation, as well as NIR thermal energy in the powder bed, a rotational mixer was used simultaneously with combined NIR-UV treatment in this study. Simultaneous mixing could increase the contact area of radiation on powder particles and facilitate the use of NIR-UV radiant treatment for controlling *C. sakazakii* in dry infant formula.

To date, there are no published reports dealing with the effectiveness of NIR and UV-C combination treatment against pathogenic bacteria in powdered foodstuffs. For this reason, the results of this study can only be compared with the results obtained from our previous study (24), which dealt with a different powdered food matrix (red pepper powder) and different microorganisms (*S.* Typhimurium and *E. coli* O157:H7). The temperature increase rate of the infant formula powder core was similar to that of red pepper powder. However, a longer treatment time and higher core temperature (7 min and 73°C) were required than for previously studied pathogens (5 min and 62°C) to obtain the same (ca. 3-log-unit) reduction in infant formula powder. These results might be attributed to the higher resistance of *C. sakazakii* than of other *Enterobacteriaceae* under dry conditions. *C. sakazakii* in the stationary phase can produce yellow pigments (carotenoids) known to stabilize cellular membranes, influence cellular membrane fluidity, and scavenge reactive oxygen species and to be responsible for its survival and behavior in stressful environments (30). Furthermore, the increased amount of fat, protein, and carbohydrate in infant formula powder may also protect *C. sakazakii* against NIR-UV treatment. Therefore, the optimized treatment conditions of a combined NIR-UV system could vary depending on the target pathogenic microorganisms and the model food being investigated.

In order to validate the application of this new bactericidal technology, it is essential to study the ongoing microbiological dynamics of the processed food. Sublethally injured cells could assume added significance, since they are able to be resuscitated and to regain their pathogenicity under suitable conditions (31). Therefore, cell populations enumerated directly on selective media following treatment are likely not representative of the total surviving cells in infant formula powder. In this study, there were no significant ($P > 0.05$) differences in reduction levels determined by plating on selective agars with and without a resuscitation step at all treatment time intervals (Table 1). This suggests that simultaneous NIR-UV treatment effectively inactivated *C. sakazakii* in powdered infant formula without generating many injured cells that potentially could recover.

In our previous study, the mechanism of the synergistic lethal effect of combined NIR-UV treatment was investigated, and we confirmed that damage to the bacterial cell envelope was the main factor related to the synergistic lethal effect (24). In the present study, the PI uptake test was utilized to determine the inactivation mechanism of *C. sakazakii*. This method was evaluated in an investigation involving *S.* Typhimurium and *E. coli* O157:H7 (24). PI emits fluorescence when binding to nucleic acids and does not pass through intact cell membranes (32). As shown in Table 2, the value of PI uptake induced by the simultaneous application of NIR-UV was significantly higher than the sum of PI uptake values reached by separate NIR and UV treatments, and this tendency was consistent with the synergistic log reduction data obtained from selective media (Table 1). These results were in accordance with our previous work and provide clear evidence for the inactivation mechanism underlying the synergistic effect of combined NIR-UV treatment for *C. sakazakii*.

According to the FDA's definition, an agent that provides a minimum 2-log-unit microbial reduction is accepted as an antimicrobial technology by the food industry (20). Our results revealed that about a 3-log-unit reduction in the number of *C. sakazakii* cells in powdered infant formula can be achieved by simultaneous NIR-UV treatment within 7 min of exposure. However, considering the very low observed level (0.36 to 0.66 CFU/100 g) of *C. sakazakii* contamination in powdered infant formula (6), NIR-UV treatment at a moderate intensity would virtually ensure that a serving would not contain this enteric pathogen and could be applied practically by the dairy industry. Although the pilot instrument used in the current study was batch type and the capacity was comparatively small, this bactericidal system for powdered foods could easily be expanded to practical industrial scale by utilizing it in the form of continuous line processing.

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