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RESEARCH NOTE

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Inactivation of *Enterobacter sakazakii* Inoculated on Formulated Infant Foods by Intense Pulsed Light Treatment

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Abstract *Enterobacter sakazakii* is a representative microorganism whose presence in infant foods can cause serious disease. The purposes of this study were to determine the inactivation effects of intense pulsed light (IPL) on *E. sakazakii* and the commercial feasibility of this sterilization method. The inactivation of *E. sakazakii* increased with increasing electric power and treatment time. The cells were reduced by 5 log cycles for 4.6 and 1.8 msec of treatment at 10 and 15 kV of electric field strength, respectively. The sterilization effects on commercial infant foods were investigated at 15 kV. The cell population in an infant beverage, an infant meal, and an infant powdered milk product inoculated with *E. sakazakii* were inactivated exponentially as a function of time and reduced by 4.0, 2.5, and 1.5 log cycles for 9.4, 7.0, and 7.0 msec of treatment time, respectively.

Keywords: *Enterobacter sakazakii*, inactivation, infant food, intense pulsed light (IPL), sterilization

Introduction

Commercial infant foods contain various nutrients that stimulate the growth of infants, and these nutrients can also support the growth of microorganisms. Since infants are especially susceptible to food poisoning, the microbiological safety of infant foods requires special consideration. Recently there have been frequent reports of the presence of bacteria in infant foods, which has led to consumer concern about the safety of such food. Conventional thermal sterilization has been applied to most infant foods, but it can reduce the levels of essential nutrients and degrade the sensory qualities. Infant powdered products require special care since they are not sterilized (1). Moreover, despite some commercial infant formulas being subject to heat treatment, *Enterobacter sakazakii* can still be isolated from these products (2). Although the incidence of infection by *E. sakazakii* is low, it can induce life-threatening, meningitis, septicemia, and necrotizing enterocolitis in infants. Therefore, it is regarded as especially risky to premature, low-birth-weight, or immunocompromised infants, and infants younger than 28 days (3).

Intense pulsed light (IPL) is currently being recommended as an innovative nonthermal sterilization technology that kills microorganisms using short pulses of intense broad-spectrum electromagnetic radiation (4,5). Various terms have been used to describe it, including pulsed ultraviolet (UV) light, high-intensity broad-spectrum pulsed light, pulsed-UV disintegration, pulsed light, and pulsed white light. This nonthermal technology is designed to produce stable and safe food products that are not affected except for damage induced by heating (5). Pulsed light results in very few residual compounds and does not involve the use of chemicals that cause environmental pollution or harm humans. Moreover, since a xenon lamp used in this study does not contain mercury, it is also more eco-friendly than a UV lamp (6). The light irradiation used for pulsed-light treatment contains wavelengths from the UV to the near-infrared region. At least 70% of the electromagnetic energy falls within the wavelength range from 170 to 2,600 nm, and it is very similar to sunlight having a peak emission between 400 and 500 nm (4,5,7). The UV region, especially UV-C, is very important for microbial inactivation, and IPL includes about 25% of the UV spectrum (5,8). Although various spectra, durations, and intensities of pulsed light have been considered for sterilizing foods and packaging, this technology currently focuses on broad-spectrum (i.e., 'white') light flashes (4).

The objectives of the present study were to explore the inactivation of *E. sakazakii* by IPL as a function of treatment voltage and time and the commercial feasibility of IPL sterilization method to formulated infant foods including an infant beverage, an infant meal, and infant powdered milk.

Materials and Methods

Microorganisms and cultivation condition *Enterobacter sakazakii* ATCC 51329 was obtained from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) and cultured on tryptic soy agar (TSA; Difco, Livonia, MI,

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Table 1. Composition of formulated infant foods and infant powdered milk used in the present study

Component	Infant beverage (%)	Infant meal (%)	Infant powdered milk (%)
Carbohydrate	7	14	56
Fat	0	0	27
Protein	0	0	12
Ash	0	0	2.5
Vitamin C	0.01	0.02	0
Oligosaccharide	1	0	0
L-Carnitine	0.002	0	0
Water	91.988	85.98	2.5
Total	100	100	100

USA). One or 2 colonies were picked from the agar plate and inoculated into a test tube containing 5 mL of tryptic soy broth (TSB), and then precultured for 24 hr at 37°C. A 1 mL aliquot of precultured fluid was inoculated into a Erlenmeyer flask containing 100 mL of TSB and cultured for 8 hr, at which point the cells had grown up to the asymptotic logarithmic phase.

Infant foods The infant foods used in this study were commercially formulated infant foods and infant powdered milk. The formulated infant foods comprised an infant beverage (Ion beverage; Il-Dong, Seoul, Korea) and an infant meal (Gerber; Nestle, Vevey, Switzerland) and the infant powdered milk was manufactured by Nam-Yang (Seoul, Korea). Their chemical compositions are listed in Table 1.

IPL treatment on *E. sakazakii* To investigate the inactivation effect of IPL treatment on *E. sakazakii* in plates, the prepared sample was spread onto a TSA plate at the predicted dilution, and then the sample was treated with IPL under the following conditions: 10-15 kV, 15 Hz, 0-10 msec, and with the sample surface 60 mm from the lamp. To investigate the inactivation effect of IPL treatment on the strain in formulated infant foods, the culture fluid was inoculated into infant foods. The initial spoilage of microorganisms was 10^5 CFU/g. To test the IPL inactivation effect in infant powdered milk, the freeze-dried microorganism was mixed with infant powdered milk to an initial density of 10^5 CFU/g. Infant foods were spread to a thickness of 2 mm on a petri dish and treated with IPL. The treatment conditions were 15 kV, 10 Hz, 0-12 msec, and with the sample surface 60 mm from the lamp.

Device for generating IPL A system to generate pulsed light was designed and manufactured in our laboratory. The system consists of control, lamp, and power-supply sections (Fig. 1). The electricity source can generate a maximum voltage of 30 kV, with the direct current (DC) and control portions adjusting the frequency and width of the pulse. The input 220 V alternating current (AC) supply source at 25 A is rectified and transformed to a maximum permissible voltage of 25 kV and supplied to a 0.12-μF capacitor via a 6-MΩ series resistor. The electricity is stored using resonance charging, and a thyratron rated at

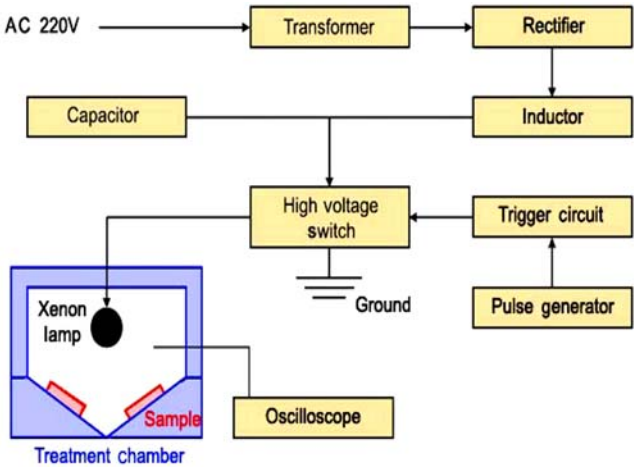

Fig. 1. Schematic diagram of the intense pulsed light (IPL) treatment system.

25 kV and 1,000 A is used as the switch for momentary discharge. The quartz lamp used to generate IPL in this study (XAP Series type NL 4006; Heraeus Nobelight Ltd., Cambridge, UK) was filled with xenon at a pressure of 600 hPa, and was 145 mm long with an outer diameter of 7.14 mm. The waveform fed to the lamp was viewed on an oscilloscope (Model 9300 AM; Dual 400 MHz, Lecroy Digital Oscilloscope, Geneva, Switzerland), which indicated that it was an exponentially decaying pulse.

Viability of microorganisms Infant foods treated with IPL were spread onto a TSA plate at the predicted dilution and cultured for 48 hr at 37°C. The surviving fraction (S) was calculated at the number of the strain surviving after IPL treatment (N) relative to the initial number of the strain (N_0).

Statistical analysis All inactivation tests were conducted in triplicate. Data were expressed as the means of these values±standard deviations (SD) calculated using Microsoft Excel 2007 statistical analysis algorithms (Microsoft, Redmond, WA, USA), with their significant differences ($p<0.05$).

Results and Discussion

Inactivation of *E. sakazakii* in plates by IPL treatment The inactivation effects of IPL treatment on *E. sakazakii* as functions of light intensity and treatment time are shown in Fig. 2. The viability cells of microorganisms decreased with increasing treatment time and as the voltage increased from 10 to 15 kV. The cell reduction was 1.0, 3.5, and 5.0 log cycles for 1.0, 3.0, and 4.6 msec of treatment at 10 kV, respectively, and was 1.5, 3.0, and 5.0 log cycles for 0.6, 1.2, and 1.8 msec of treatment at 15 kV, respectively. Also, these results indicate that these microorganisms were inactivated exponentially as a function of time.

The inactivation of microorganisms by pulsed light is influenced by the light intensity, treatment time, wavelength, type of microorganism, type of food, and the distance between the lamp and sample (9), with the light intensity having the greatest effect. Previous studies have also

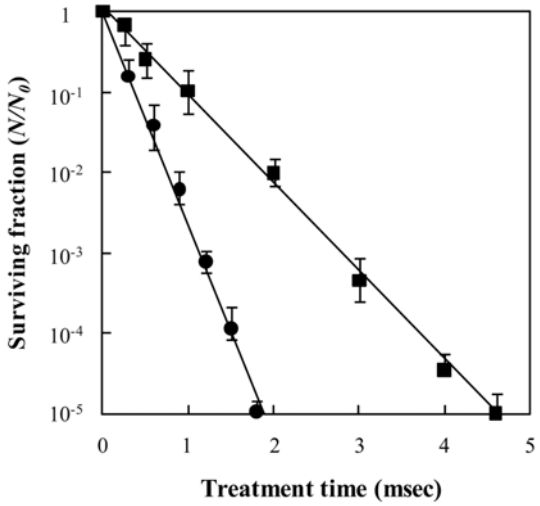

Fig. 2. Inactivation of *E. sakazakii* as functions of IPL intensity and treatment time. ■, 10 kV; ●, 15 kV. Data points were shown as the mean±SD (n=3).

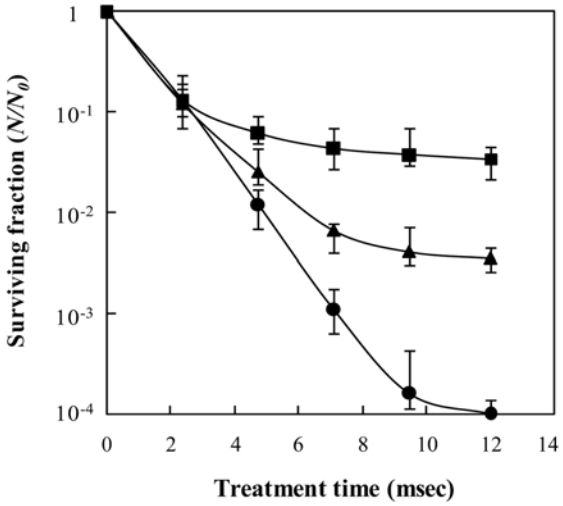

Fig. 3. Inactivation of *E. sakazakii* in various types of formulated infant foods as functions of IPL treatment time at 15 kV. ●, Infant beverage; ▲, infant meal; ■, infant powdered milk. Data points were shown as the mean±SD (n=3).

shown that the inactivation of microorganisms by IPL varies with the electric power and treatment time. Cho *et al.* (10) studied the inactivation effects by IPL on lactic acid bacteria for 0-2,500 μsec of treatment at 15-25 kV. They reported an exponential relationship between the electric power and inactivation of microorganism, as evident in the present study. Jun *et al.* (11) also found this relationship after treating *Aspergillus niger* spores inoculated into a corn meal suspension, with the inactivation of *A. niger* increasing from a 1.352 log reduction to a 4.954 log reduction when the electric power was increased from 2.0 to 3.8 kV, and from a 0.176 to a 1.352 log reduction when the treatment time increased from 20 to 100 sec for treatment at 2.0 kV. These results indicate that inactivation increases with both the electric power and treatment time.

Inactivation of *E. sakazakii* in formulated infant foods by IPL treatment The sterilization effects of IPL treatment on commercially formulated infant foods inoculated with *E. sakazakii* were investigated at 15 kV. As shown in Fig. 3, the order of inactivation by IPL treatment was as follows: infant beverage, infant meal, and infant powdered milk. The cell reduction in the infant beverage was 1.0, 2.0, and 4.0 log cycles for 2.3, 4.7, and 9.4 msec of treatment at the same voltage, respectively, and the death rate increased exponentially with treatment. In case of the infant meal (Gerber, Parsippany, NJ, USA), the reduction in *E. sakazakii* was 1.0 and 2.5 log cycles for 2.3 and 7.0 msec of treatment, respectively, and increased exponentially with the treatment time until 7.0 msec. In the infant powdered milk mixed with freeze-dried *E. sakazakii*, the cell population reduced by 1.0 and 1.5 log cycles for 2.3 and 7.0 msec of treatment, respectively, but it did not make a significant difference for longer treatment times.

The results of this study elucidate that the sterilization efficacy of the IPL depends on the moisture content of the product (Fig. 3, Table 1). Several previous studies have shown that pulsed-light treatment was very effective at inactivating microorganisms in liquid foods. Dunn *et al.* (4) reported that pulsed light exerted strong inactivation effects on water inoculated with several pathogenic bacteria. Hillegas and Demirci (12) reported that pulsed-UV light inactivated 89.4% of *Clostridium sporogenes* bacteria in clover honey. PurePulse Technology (San Diego, CA, USA) reported on sterilization by pulsed light in 1999. They filled a chamber with 20%(w/v) glucose and distilled saline water or water, inoculated it with microorganisms, and then treated it with pulsed light. Their results showed very effective inactivation of microorganisms in some beverages, and predicted that the sterilization of beverages by pulsed light would become a very useful technology. Factors that affect the inactivation of microorganisms in liquid food include its color, viscosity, the depth of the sample, transparency, and the nutrients therein.

In this study we found that the inactivation in the infant meal (Gerber) of the formulated infant foods was effective but lower than that in the infant beverage. This might be attributable to the moisture content or the viscosity of the infant meal Gerber reducing the penetration of pulsed light (which is responsible for the difference in the transfer of pulsed light energy throughout the foods), and the opaqueness or the dark color of Gerber reducing the absorption of the light (which is responsible for the destruction of cell membranes and DNA, and the elution of protein). Whilst pulsed light can theoretically be applied to powdered foods, the present study was the first to actually do this. The results to date indicate that IPL can be used to sterilize powdered food, but that further investigations are required for dried foods in order to accomplish successful industrial applications.

There has been insufficient investigation of the application of IPL to food sterilization. This study is highly significant since it is the first related to the application of IPL to foods in Korea. This study confirmed the effective inactivation of *E. sakazakii* by IPL treatment and the possible commercial usefulness of IPL in sterilizing liquid, paste, and powdered foods. Pulsed-light treatment for foods was approved by

the US FDA in 1996, but its safety and effects on the sensory quality of food are unclear. Therefore, future studies should investigate the application of pulsed light on a commercial scale, since this putatively represents a very important technology for nonthermal sterilization.

Acknowledgments

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