Monitoring Ultraviolet (UV) Radiation Inactivation of *Cronobacter sakazakii* in Dry Infant Formula Using Fourier Transform Infrared Spectroscopy

Qian Liu, Xiaonan Lu, Barry G. Swanson, Barbara A. Rasco, and Dong-Hyun Kang

Abstract: *Cronobacter sakazakii* is an opportunistic pathogen associated with dry infant formula presenting a high risk to low birth weight neonates. The inactivation of *C. sakazakii* in dry infant formula by ultraviolet (UV) radiation alone and combined with hot water treatment at temperatures of 55, 60, and 65 °C were applied in this study. UV radiation with doses in a range from 12.1 ± 0.30 kJ/m^2 to 72.8 ± 1.83 kJ/m^2 at room temperature demonstrated significant inactivation of *C. sakazakii* in dry infant formula ($P < 0.05$). UV radiation combining 60 °C hot water treatment increased inactivation of *C. sakazakii* cells significantly ($P < 0.05$) in reconstituted infant formula. Significant effects of UV radiation on *C. sakazakii* inactivation kinetics (*D* value) were not observed in infant formula reconstituted in 55 and 65 °C water ($P > 0.05$). The inactivation mechanism was investigated using vibrational spectroscopy. Infrared spectroscopy detected significant stretching mode changes of macromolecules on the basis of spectral features, such as DNA, proteins, and lipids. Minor changes on cell membrane composition of *C. sakazakii* under UV radiation could be accurately and correctly monitored by infrared spectroscopy coupled with 2nd derivative transformation and principal component analysis.

Keywords: chemometrics, *C. sakazakii*, infrared spectroscopy, UV radiation

Introduction

Cronobacter sakazakii (*Enterobacter sakazakii*) is an opportunistic pathogen and causes meningitis and necrotizing entercolitis in low birth weight neonates with reported fatality rates of 50% to 80% (Iversen and Forsythe 2003). *Cronobacter sakazakii* was first identified in 1980 and then reclassified into several genomospecies (Iversen and others 2008). Although the reservoir for *C. sakazakii* is unknown in many cases, dry infant formula is a source and vehicle of infection. Dry infant formula is a nutritional analogue to human breast milk. *Cronobacter sakazakii* is introduced into dry infant formula either by adding contaminated ingredients during manufacture or by handling and packaging after spray drying. The ability of *C. sakazakii* to adapt to dehydration stress and survive in dry infant formula may be associated with the production of yellow pigments and colonic acid production (Scheepe-Leberkuhne and Wagner 1986; Johler and others 2010). The primary source of *C. sakazakii* in hospitals and household may be from contaminated hospital equipments, including incubators, blenders, and utensils using for preparation (Chen and others 2009). Environmental control and raw material hygiene are the main method to reduce the prevalence of contamination in dry infant formula by *C. sakazakii*. Because controlling of *C. sakazakii* in dry infant formula is complicated and difficult, it is necessary to develop more effective control methods to inactivate it in dry formula. The new method should be cost effective and its germicidal characteristics should enable its application in postpasteurization processing in dry infant formula manufacture.

The 253.7 nm ultraviolet (UV) radiation is germicidal because of its unique ability to penetrate cell wall of microorganism and damage DNA (Quitero-Ramos and others 2004). The 253.7 nm UV radiation inactivates microorganisms by inducing formation of pyrimidine dimers that distort the DNA helix and block microbial cell replication.

The inactivation effect of UV radiation on *C. sakazakii* in dry infant formula has not been reported so far, which is one of the purposes in the current study. In addition, the inactivation mechanism is explored using Fourier transform infrared (FT-IR) spectroscopy (4000 to 400 cm^{-1}). FT-IR spectroscopy is form of vibration spectroscopy and has been employed to characterize bacteria to species/strains level on the basis of the macromolecular composition of the cell walls and membranes (Puppels and others 1990; Naumann and others 1991, for review see Maquelin and others 2002). Many works only employed pure system (such as water) rather than food matrices due to tremendous interference of matrix constituents on spectral features. Several novel techniques such as filtration (Lu and others 2011) and immunomagnetic beads (Yu and others 2006; Ravindranath and others 2008; Davis and others 2010a) have been developed to separate bacteria from complicated food matrices. In current study, we used filtration to separate *C. sakazakii* from dry milk powder to

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eliminate the problem of spectral interference. This technique was recently validated to be better than the use of immunomagnetic bead (Davis and others 2010b), resulting in high selectivity and sensitivity.

In this study, the efficacy of UV radiation for inactivating *C. sakazakii* in dry infant formula was evaluated. UV radiation only and in combination with 55, 60, and 65 °C hot water treatments were also applied to inactivate *C. sakazakii* in infant formula. The mechanism of inactivation was investigated by FT-IR spectroscopy.

Materials and Methods

Bacterial strains, culture methods, and preparation of stock cultures

Three strains of *C. sakazakii* (American Type Culture Collection [ATCC] 51329, 29544, and 12868) were obtained from the School of Food Science culture collection at Washington State Univ. (Pullman, Wash., U.S.A.). All bacteria strains were stored at −80 °C in tryptic soy broth (TSB, Difco Laboratories, Detroit, Mich., U.S.A.) with 15% glycerol. Frozen cultures of the 3 strains were streaked onto tryptic soy agar (TSA, Difco Laboratories) and incubated for 24 h at 37 °C. The grown cultures were stored at 4 °C for routine use. Cultures of each strain were combined together to construct a culture cocktail and harvested by centrifugation at 4000 rpm (centra CL2, 4675 × *g*, West Chester, Pa., U.S.A.) for 20 min at room temperature. Cultures were washed twice with buffered peptone water (Difco). The pellets were resuspended in buffered peptone water, corresponding to 10^8 to 10^9 CFU/mL.

Dry infant formula sample preparation and inoculation

Dry infant formula (Enfamil, Mead Johnson & Co., Evansville, Ind., U.S.A.) was purchased from a local grocery store. Five grams of dry infant formula powder was placed into a Petri dish (150 × 15 mm, Becton Dickinson & Co., Franklin Lakes, N.J., U.S.A.) and spread into a layer of *ca* 0.032 mm. One hundred microliters of the cocktail was inoculated to dry infant formula powder by depositing droplets in 5 locations with a micropipette. The dry infant formula was dried in a laminar flow biosafety hood with the fan running for 30 min. After drying, clumps of inoculated infant formula powder were crushed with a sterile spatula and thoroughly shaken to produce a homogeneous dispersal of inoculums throughout the dry infant formula.

UV radiation treatment

The UV light chamber for treatment of dry infant formula was custom built at the School of Food Science, Washington State Univ. (Pullman). The chamber contains two 45.4-cm-long UV lamps 10 cm apart (SunRay Technologies, Inc., Killington, Vt., U.S.A.). The lamps are suspended across the chamber with a distance of 15 cm from the base of the chamber. UV intensity at 253.7 nm was determined by a UV radiometer (Steril-Aire, Burbank, Calif., U.S.A.) at the surface of the Petri dish. The radiometer sensor was placed under the UV lamp with the lamp on for 30 min to obtain a consistent reading. The interior of chamber is lined with a highly reflective metal foil (SunRay Technologies) to increase UV intensity and to minimize any shadowing effect on irregular surface shaped dry infant formula samples. Plates of inoculated dry infant formula were individually subjected to UV treatment. The intensity was kept constant and selected exposure times were applied to allow respective doses (Table 1). The Petri dish was shaken for 30 s to obtain a homogeneous exposure of dry infant formula granules to UV radiation.

Combined UV radiation and hot water treatment

Five grams of inoculated and air-dried infant formula was placed into a Petri dish and spread into a thin layer. After exposure to UV radiation for 20 min at room temperature, UV radiation exposed infant formula was reconstituted in 10 mL sterile distilled water at room temperature in a stomacher bag and homogenized for 2 min with a Seward stomacher (Stomacher 80, Seward, London, U.K.). Hot water treatment was conducted in a water bath (VWR Scientific, West Chester) for selective time intervals at 55, 60, and 65 °C. The temperature of the dry infant formula samples was monitored by a type omega thermocouple (OMEGA Engineering Inc., Stamford, Conn., U.S.A.) and temperature controlled within ±0.5 °C. The treatment time was controlled by a stopwatch (Thermo Fisher Scientific Inc., Waltham, Mass., U.S.A.). The reconstituted infant formula was removed from the water bath and immersed in ice immediately after each treatment to stop heating.

Bacterial enumeration

UV-treated dry infant formula powder was rehydrated as described previously. One milliliter sample aliquots of reconstituted infant formula were serially diluted 10-fold in 9 mL of sterile 0.2% peptone water (Difco) and 0.1 mL of samples were spread-plated onto Petri plates of OK Medium (OK; Acumedia, Lansing, Mich., U.S.A.), a medium for selective recovery and enumeration of *C. sakazakii*. The bacteria were also enumerated on to TSA to recover the injured cells for the combined treatments of UV radiation and hot water. Agar plates were incubated at 37 °C for 24 h and colonies were enumerated. Colonies fluorescing when illuminated with 365 nm UV light were counted (Oh and Kang 2004).

Membrane filtration and FT-IR spectroscopy

Nonfat milk powder was used as a surrogate for dry infant formula for UV treatments to eliminate the background noise raised by large fat molecules. Two one hundredths of a gram of nonfat milk was dissolved in 50 mL of sterile saline water (0.85% w/v) and filtered through an aluminum oxide membrane filter (0.2 μm pore size, 25 mm optical diameter) (Anodisc, Whatman Inc., Clifton, N.J., U.S.A.) using a Whatman vacuum glass membrane filter holder (Whatman Catalog nr 1960-032) to harvest bacterial cells. The anodisc filters were removed from the filtration apparatus and air-dried under laminar flow at room temperature for 10 min to allow a homogeneous film of bacterial cells to form.

FT-IR spectra were collected using a Nicolet 380 FT-IR spectrometer (Thermo Electron Inc., San Jose, Calif., U.S.A.). The aluminum oxide membrane filter coated with a uniform and thin

Table 1–Survival population of *C. sakazakii* in dry infant formula after UV radiation treatment ($N = 3$).

UV radiation treatment time (min)	0	5	10	15	20	25	30
UV radiation dose (kJ/m^2)	N/A	12.1 ± 0.30	24.3 ± 0.61	36.4 ± 0.91	48.6 ± 1.21	60.7 ± 1.52	72.8 ± 1.82
Survival population (log CFU/g)	7.26 ± 0.05[a*]	6.58 ± 0.10[b]	6.52 ± 0.19[c]	6.44 ± 0.09[d]	6.17 ± 0.14[e]	5.88 ± 0.22[f]	5.92 ± 0.43[g]

*Values followed by different lower case letters are statistically different ($P < 0.05$).

layer of bacterial cells was placed in direct contact with the diamond crystal cell (30000 to 200 cm^{-1}) of attenuated total reflectance (ATR) detector. Infrared spectra were recorded from 4002 to 399 cm^{-1} at a resolution of 8 cm^{-1}. Each spectrum was acquired by adding together 32 interferograms. Five spectra were acquired for intact and UV-treated *C. sakazakii* at different locations on the aluminum oxide filter for a total of 15 spectra for each group of cells. Triplicate experiments ($N = 3$) were conducted and spectra from the first 2 experimental runs were used to establish chemometric models while the spectra from the 3rd experiment were used for model validation.

Data preprocessing and chemometrics

Infrared spectra were first preprocessed by EZ OMNIC 7.1 a (Thermo Electron Co., Waltham, Mass., U.S.A.). Relevant background was subtracted from each raw spectrum. Automatic baseline correction was employed to flatten the baseline, following by smooth of 5 (Gaussian function of 9.643 cm^{-1}). The preprocessed spectra were read by Matlab 2010, version a (Math Works Inc., Natick, Mass., U.S.A.) with .xls format by Excel (Microsoft Inc., Redmond, Wash., U.S.A.). The reproducibility of vibrational spectra from 3 independent experiments ($N = 3$) was investigated by calculating D_{y1y2} according to Eq. (1) and (2) (Moen and others 2005). In the equations, y_{1i} and y_{2i} are signal intensities of 2 selected spectra while $\bar{y}_1$ and $\bar{y}_2$ are mean values of signal intensities of 2 selected spectra; n represents the data points in the selected wavenumber region. The D_{y1y2} ranges from 0 to 2000 and small values define good reproducibility of spectra. Zero means the 2 spectra are identical; 1000 means the 2 spectra are totally unrelated. Two thousand means the two spectra are negatively related.

$$r_{y1y2} = \frac{\sum_{i=1}^{n} y_{1i} y_{2i} - n\bar{y}_1\bar{y}_2}{\sqrt{\sum_{i=1}^{n} y_{1i}^2 - n\bar{y_1}^2}\sqrt{\sum_{i=1}^{n} y_{2i}^2 - n\bar{y_2}^2}} \quad (1)$$

$$D_{y1y2} = (1 - r_{y1y2})\,1000. \quad (2)$$

Second derivative transforms (with a gap value of 10 cm^{-1}) were performed for spectral processing in Matlab. Principal component analysis (PCA) was employed. PCA is an unsupervised chemometric tool to reduce the dimensionality of multivariate data while preserving most of the variances and provides a 2-dimensional (2-D) or 3 dimensional cluster of results for group segregation (Lu and others 2010). The wavenumbers between 1800 cm^{-1} and 900 cm^{-1} were selected for infrared spectroscopic-based chemometric analyses. This wavenumber region contains many useful absorption bands that are related to specific functional groups derived from molecules involved in analytes.

Statistical analysis

All experiments were repeated 3 times with duplicate samples. Significant differences ($P < 0.05$) between microbial levels under selected treatments were determined by 1-way analysis of variance followed by *t*-test using Matlab.

Results and Discussion

UV radiation

UV radiation with a range from 12.1 kJ/m^2 to 72.8 kJ/m^2 imparted significantly germicidal effect ($P < 0.05$) to *C. sakazakii* in dry infant formula (Table 1). UV radiation for 25 min treatment of dry infant formula was the most effective treatment with a 1.38 log_{10} CFU reduction per gram. A curve of *C. sakazakii* survival population versus UV radiation treatment time was plotted (Figure 1). An initial rapid inactivation in the first 5 min followed by a period of rapid inactivation at 25 to 30 min was observed. The initial rapid inactivation resulted from the UV radiation exposure and the 2nd rapid inactivation may result from the raised temperature in the chamber after 20 min. After 20 min of UV radiation treatment, the temperature in UV chamber was about 37 °C. Complete inactivation was not achieved because the UV radiation did not completely penetrate the dry infant formula. The germicidal effect of UV radiation is influenced by food matrices and the physiological state of bacteria (Koutchma 2008). Although UV radiation is well established for sanitation of air, water, liquid food pasteurization, and surface decontamination, UV radiation of powdered food is still under investigation. U.S. Food and Drug Administration (FDA) recommendations stated that to achieve a 4-log microbial inactivation, the UV radiation exposure must be at least 400 J/m^2 for all parts of the product (U.S. FDA 2009). In this experiment, a 25 min UV radiation treatment led to a radiation dose of *ca* 60.7 kJ/m^2 (Table 1). The radiation dose required for liquid food listed by FDA is too small for food powders. Compared to water, powdered food exhibits a range of optical and physical properties and diverse chemical compositions that influence UV transmittance, dose delivery, momentum transfer, and consequently microbial inactivation (Koutchma 2008). The transmittance of UV radiation in dry infant formula is much smaller comparing to transmittance in liquid food. Infant formula particles absorb and scatter UV radiation due to chemical compositions, optical properties, and shape. Moreover, the clumps formed during inoculation can provide a site for the aggregation of bacteria in the core of the clump surrounded by infant formula particles (Koutchma 2008). The clumps can protect *C. sakazakii* cells from UV radiation. Sensitivity of microorganisms to UV radiation also influences the efficacy of bacteria inactivation. Cell wall structure, thickness and composition, absorbent compounds, and nucleic acid structure are important to bacterial sensitivity exposed to UV radiation (Koutchma 2008). *Cronobacter sakazakii* in the stationary phase can produce carotenoids known to stabilize cellular membranes, influence cellular membrane fluidity, scavenge reactive oxygen species, and play a role in the survival of

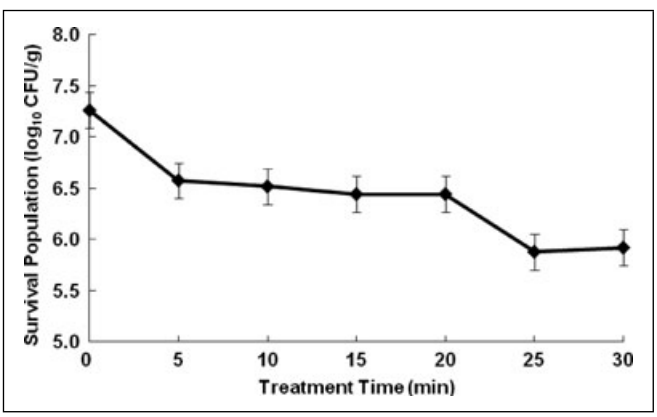

Figure 1–Microbial survival curve of *C. sakazakii* inoculated in dry infant formula and treated with UV radiation (253.7 nm) for 5 min, 10 min, 15 min, 20 min, 25 min, and 30 min. Points represented the mean of log_{10} CFU *C. sakazakii* cells per gram dry infant formula and vertical bars indicate standard deviation ($N = 3$).

C. sakazakii in stressful environments (Gruszecki and Strzalka 2005). Johler and others (2010) identified genes involved in pigment expression in *C. sakazakii* strain ES 5 and reported mutants that cannot produce yellow pigmentation were more sensitive to UV radiation than wild-type *C. sakazakii*. Lehner and others (2006) applied bacterial artificial chromosome approach and heterologous expression of the yellow pigment in *Escherichia coli* to elucidate the molecular structure of the genes responsible for pigment production in *C. sakazakii* ES 5. Lehner and others (2006) ascertained the carotenogenic nature of the pigment by *in situ* visible microspectroscopy and resonance Raman microspectroscopy. Besides, trehalose, which improves desiccation tolerance of *C. sakazakii*, may also contribute to UV radiation. Trehalose acts as a counterbalance to extracellular osmotic pressure, stabilizing phospholipid membranes and proteins by replacing the shell of water surrounding the membranes and proteins preventing irreversible damage of the cells (Mullane and others 2006).

In infant formula processing, cost efficient UV radiation is preferred as a postpasteurization processing to avoid *C. sakazakii* contamination from poor environment hygiene (Iversen and Frosythe 2003). Our data indicated that it may be feasible to use UV radiation to inactivate *C. sakazakii* in dry infant formula if the penetration could be improved.

Combined UV radiation and heat treatment

Inactivation effect on *C. sakazakii* in dry infant formula using UV radiation and reconstituted in 55, 60, or 65 °C sterilized hot water was evaluated. Table 2 presents *D* values of *C. sakazakii* contaminating infant formula subjected to UV radiation only, hot water treatment only, and combined UV radiation and hot water treatment. *D* value, decimal reduction time, is the time required for 1-log cycle reduction in the microbial population (U.S. FDA 2009). *D* value in this study describes the microbial population reduction at a constant UV intensity, temperature, and the combination effect of both. *D* values for *C. sakazakii* culture cocktail contaminating infant formula reconstituted with 55, 60, or 65 °C water were 3.99 ± 1.99, 0.87 ± 0.11, 1.10 ± 0.55 min, respectively (Table 2). *D* values obtained in this experiment were smaller than *D* values in other previous studies. Al-Holy and others (2009) reported D_{55} values for *C. sakazakii* ATCC 12868 and ATCC 29544 in reconstituted infant formula were 14.21 ± 3.58 and 14.83 ± 3.50 min. D_{60} values for *C. sakazakii* ATCC 12868 and ATCC 29544 in reconstituted infant formula were 0.53 ± 0.08 and 2.71 ± 0.32 min (Al-Holy and others 2009). Differences of *D* values obtained from the current study and previous studies may result from different food matrixes, physiological, and genetic properties of the bacteria. Inoculating *C. sakazakii* cocktail culture into dry infant formula and air drying may result in injury and death of desiccation sensitive cells (Edelson-Mammel and others 2005). Edelson-Mammel and Buchanan (2004) concluded that *C. sakazakii* may have a set of genetic determinants for heat resistance based on 2 distinct phenotypes presented in *D* value profile. Asakura and others (2007) studied the genetic heat resistance of *C. sakazakii*, and concluded that *infB* gene which encodes for a translation initiation factor is expressed in a higher amount in heat resistant strains than in heat sensitive

Table 2–*D* values of *C. sakazakii* contaminating reconstituted infant formula of hot water treatment only (hot water treatment) and UV radiation in combination with hot water treatment (combined UV radiation and hot water treatment) ($N = 3$).

Temperature	UV radiation treatment	Hot water treatment	UV radiation and hot water combined treatment
55 °C	5.11 ± 1.02[a*]	3.99 ± 1.99[b]	1.88 ± 1.05[b]
60 °C	5.11 ± 1.02[a]	0.87 ± 0.11[b]	1.00 ± 0.06[c]
65 °C	5.11 ± 1.02[a]	1.10 ± 0.55[b]	0.89 ± 0.10[b]

*Within each temperature level, values within a row followed by different lower case letters are statistically different ($P < 0.05$).

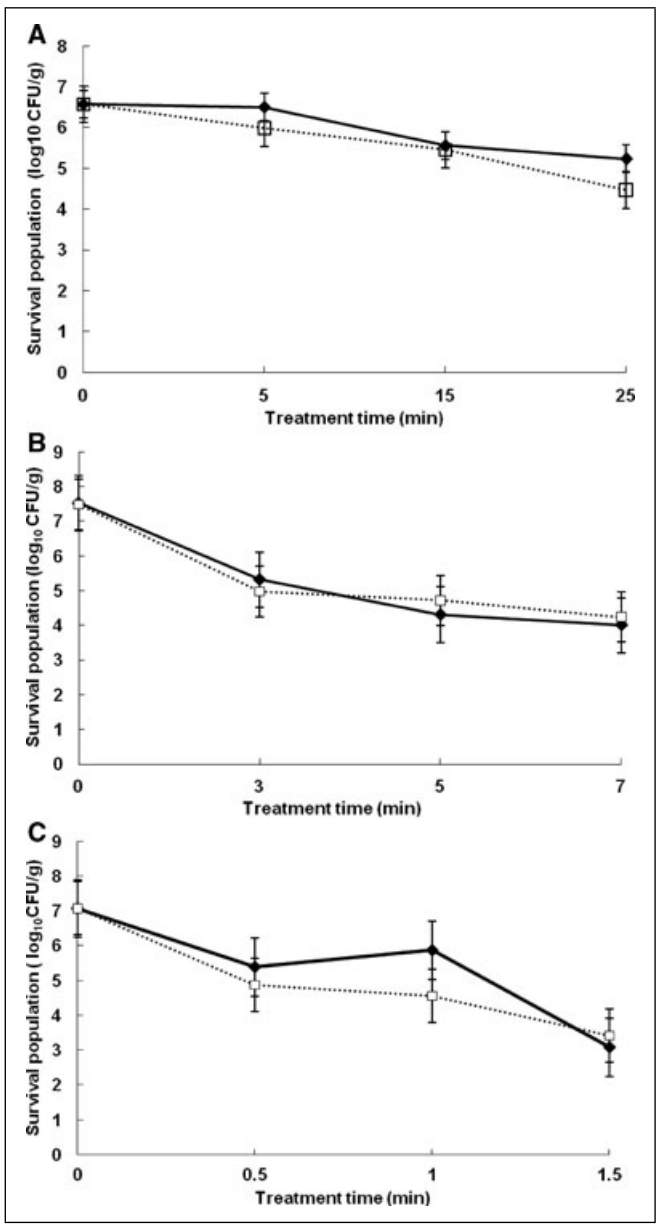

Figure 2–(A) Microbial survival population of *C. sakazakii* inoculated in dry infant formula after 55 °C hot water treatment only (black diamonds and solid line) and after UV radiation 20 min and 55 °C hot water combined treatment (white squares and dash line). Vertical bars indicate standard deviation ($N = 3$). (B) Microbial survival population of *C. sakazakii* inoculated in infant formula after 60 °C hot water treatment (black diamonds and solid line) and UV radiation 20 min and 60 °C hot water combined treatment (white squares and dash line). Vertical bars indicate standard deviation ($N = 3$). (C) Microbial survival population of *C. sakazakii* inoculated in infant formula after 65 °C hot water treatment only (black diamonds and solid line) and after UV radiation 20 min and 65 °C hot water treatment (white squares and dash line). Vertical bars indicate standard deviation ($N = 3$).

strains. *D* values for *C. sakazakii* in reconstituted infant formula after UV radiation treatments were 1.88 ± 1.05, 1.00 ± 0.06, and 0.89 ± 0.10 at 55, 60, and 65 °C (Table 2). Compared to D_{55} and D_{65} values for *C. sakazakii* in reconstituted infant formula subjected to hot water treatments and UV treatment only, D_{55} and D_{65} values decreased (Figure 2A and 2C). The decrease of *D* value indicated that the UV radiation and hot water treatment demonstrate either additive or synergistic effect on inactivation *C. sakazakii* in dry infant formula. Exposure of inoculated dry infant formula to the UV radiation probably resulted in cell injury and decrease of cellular heat resistance. Reconstituting inoculated dry infant formula in hot water destroyed

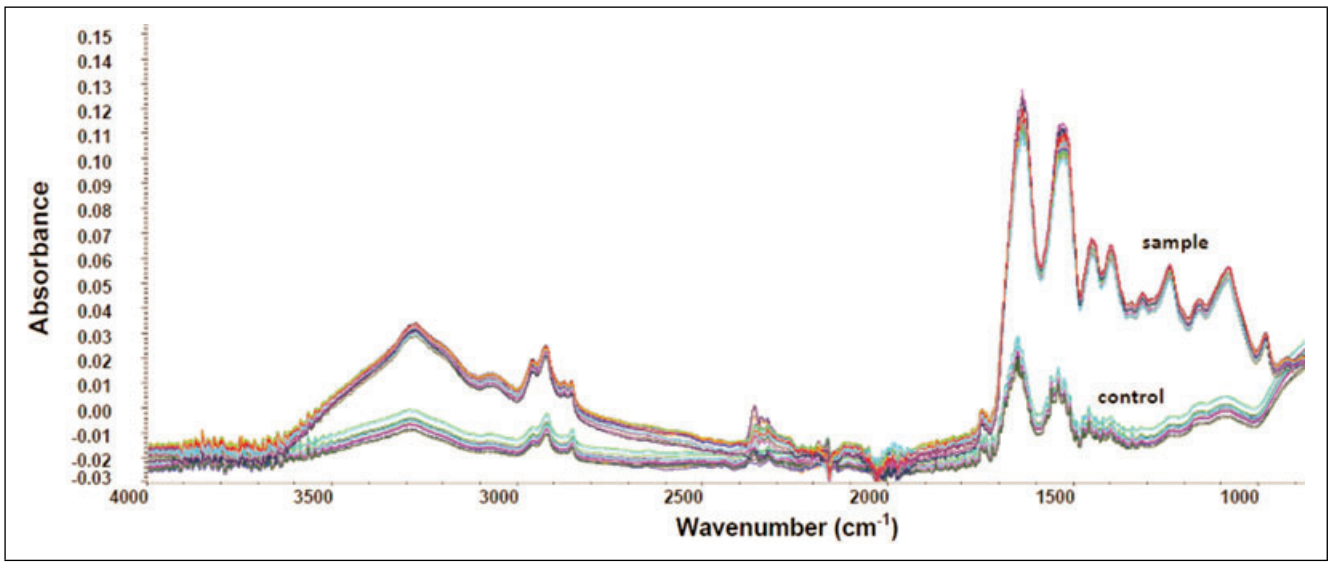

Figure 3–Raw infrared spectra of nonfat milk powder (control) and nonfat milk powder inoculated with *C. sakazakii* (sample).

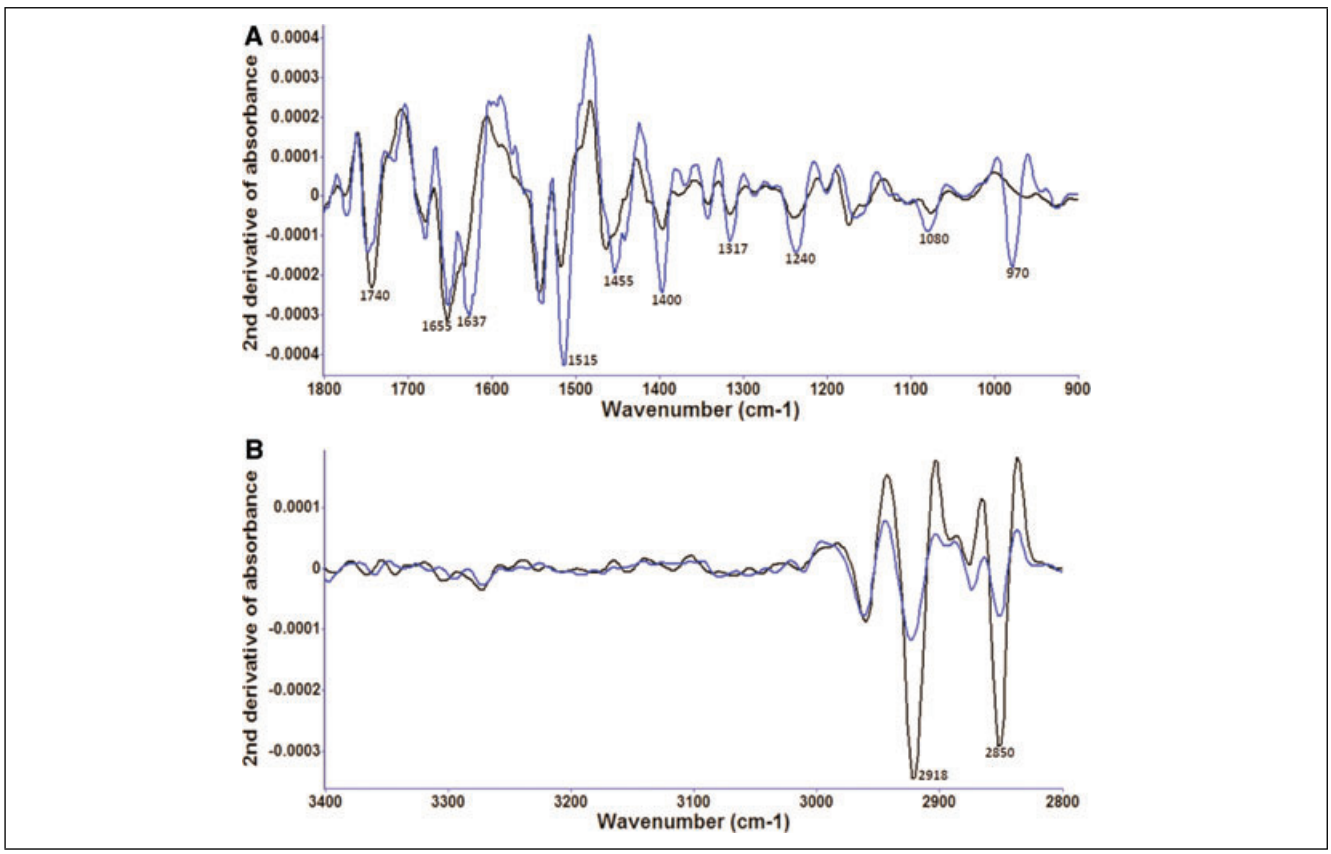

Figure 4–(A) Second derivative transforms of infrared spectra of UV-treated *C. sakazakii* (black) and untreated *C. sakazakii* (blue) between 1800 and 900 cm^{-1}. (B) Second derivative transforms of infrared spectra of UV-treated *C. sakazakii* (black) and untreated *C. sakazakii* (blue) between 3400 and 2800 cm^{-1}.

the protective powder cluster and allowed the action of heat energy.

Reconstituting dry infant formula with 70 to 90 °C water could contribute to a 4- to 6-log reduction of *C. sakazakii* (Chen and others 2009). The highest water temperature tested was lower than 70 °C and could not achieve ideal *C. sakazakii* inactivation effect before cooling down, because the maximum temperature reached after dry infant formula mixing was lower than 70 °C and was not maintained for a long enough time to inactivate the bacteria (Chen and others 2009).

Mixing hot water (higher than 70 °C) with dry infant formula could rapidly decrease the water temperature to less than 70 °C and this is ubiquitous for home preparation of infant milk (Chen and others 2009). This rapid decrease of water temperature could not provide enough inactivation effect on potential contaminated bacteria, such as *C. sakazakii*. In sum, UV radiation treatment before reconstitution may be a method to increase the hot water treatment efficacy.

FT-IR spectroscopy decoding and cluster analysis

Due to interference of fat in dry infant formula, nonfat milk powder was used for spectral analysis. The fat content of dry infant formula is 24.9% (w/w) and cannot be detected in nonfat milk powder. Traditional microbiological experiment was performed to validate no significant difference ($P < 0.05$) of bacterial inactivation by UV radiation treatment between infant formula powder and nonfat milk powder (data not shown). Figure 3 presented the raw FT-IR spectral features of nonfat milk powder and nonfat milk powder inoculated with *C. sakazakii*. Distinct differences on raw spectra were observed and provided prerequisite for subtraction control spectra from sample spectra (Lu and Rasco 2010). The intragroup variation of spectral features was significantly ($P < 0.05$) smaller than the intergroup variation of spectral features. *D* values were calculated for each group and ranged between 36.93 ± 15.13 and 76.32 ± 31.4, which demonstrated good reproducibility of spectral features of each sample.

The differences of raw FT-IR spectra for UV radiation treated and nontreated *C. sakazakii* contaminating dry infant formula were not visually discernible. Second derivative transformation of raw spectra was applied to magnify minor variability (Figure 4A and 4B). The peak at 970 cm^{-1} is assigned to the symmetric stretching mode of dianionic phosphate monoesters in cellular nucleic acids (Argov and others 2004). The peak at 1080 cm^{-1} is assigned to symmetric PO_2^- of nucleic acids (Wood and others 1998). The peak at 1240 cm^{-1} is assigned to P = O stretching (asymmetric) of $>PO_2^-$ phosphodiesters from nucleic acids (Naumann 2001). Spectral bands at 1080 and 1240 cm^{-1} reflect the functional groups information of DNA. The peak at 1317 cm^{-1} is assigned to amide III components of proteins (Yang and others 2005). The peak at 1515 cm^{-1} is assigned to amide II (Lu and others 2011). The peak at 1637 cm^{-1} is assigned to amide I of β-pleated sheet structures (Naumann 2001). The peak at 1655 cm^{-1} is assigned to amide I of α-helical structures (Lu and Rasco 2010). The amide bands provide information about α-helix, β sheet, and random coil conformations in proteins. The peak at 1400 cm^{-1} is assigned to C = O symmetric stretching of COO^- in proteins (Naumann 2001). The peak at 1455 cm^{-1} is assigned to symmetric bending modes of methyl groups in skeletal proteins (Fung and others 1996). The peak at 1740 cm^{-1} is assigned to >C = O stretching of esters (Naumann 2001). Those bands at 1317, 1400, 1455, 1515, 1637, 1655, and 1740 cm^{-1} are related to protein secondary structure (Figure 4A). The peak at 2850 cm^{-1} is assigned to C-H symmetric stretching of $>CH_2$ in fatty acids (Naumann 2001). The peak at 2918 cm^{-1} is assigned to C-H asymmetric stretching of $>CH_2$ in fatty acids (Lu and Rasco 2010). These peaks at 2850 and 2918 cm^{-1} are related to fatty acids in bacterial cell membranes (Figure 4B). Spectral variations indicated that the function of structural protein, lipid,

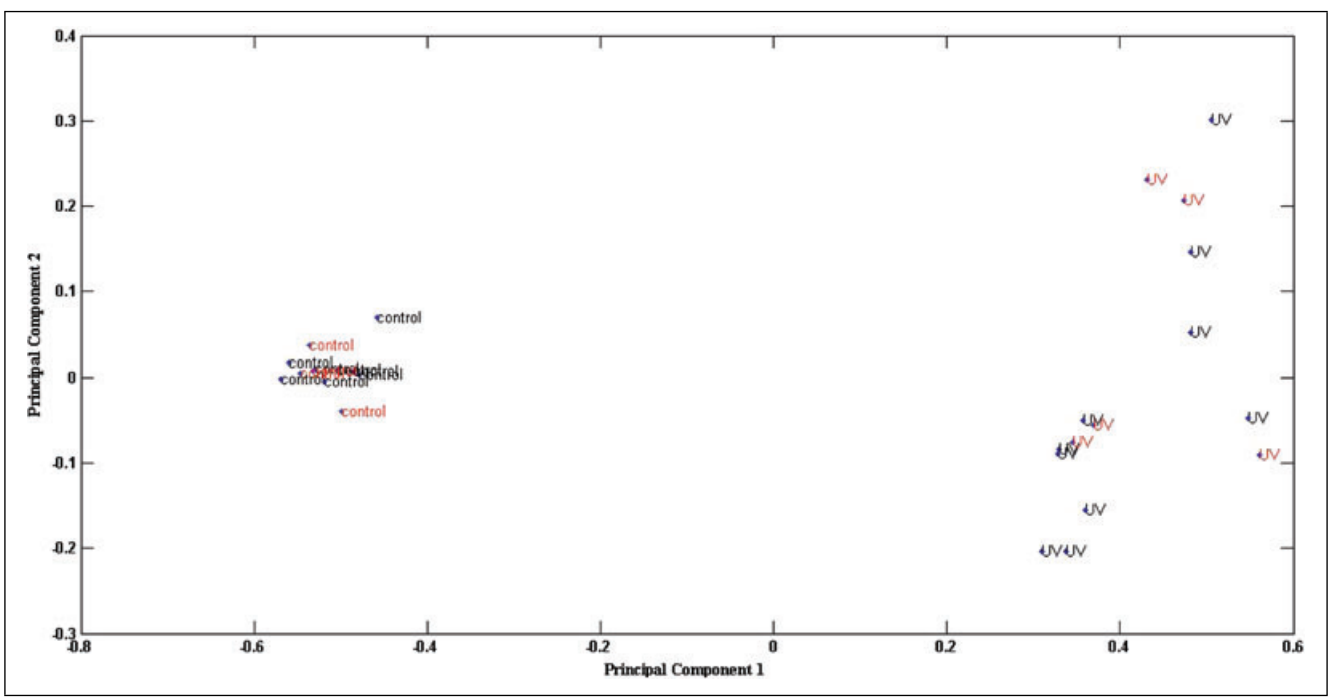

Figure 5–Principal component analysis of *C. sakazakii* treated (UV) and untreated (control) with UV radiation. The cluster analysis model was validated by new treatments (red color).

and DNA is closely associated with bacterial survival under treatment of UV radiation. In addition, the intracellular components such as nucleic acids could release out during UV radiation treatment because of cell leakage and cell death and this could be also determined by FT-IR spectroscopy. These spectroscopic results were in agreement with previous studies that formation of pyrimidine dimers could distort the DNA helix and block microbial cell replication. The significant variations in the DNA wavenumber region were determined using FT-IR spectroscopy combined with 2nd derivative transformation. Further, cell membrane structural variation was determined and major spectral variations were related to structural proteins and structural phospholipids on cell membrane.

2-D cluster analysis (PCA) was employed to segregate untreated *C. sakazakii* extracts from UV-treated extracts (Figure 5). Bacterial cells from the control treatment were tightly clustered, but variations were observed among UV treatments, indicating differences in the degree of cell injury and inactivation.

Infrared spectroscopy is useful to monitor variability in cell membrane composition that is associated with bacterial injury and survival. Lin and others (2004) discriminated intact *Listeria monocytogenes* cells from sonication-injured cells by ATR/FT-IR spectroscopy using PCA segregation clusters, noting that injury was attributed to macromolecular shearing and subsequent redistribution of cell wall components along with possible denaturation of intracellular proteins. Lu and others (2011) employed FT-IR spectroscopy coupled with loading plot analysis and PCA segregation to sort bacterial injury populations after cold and freeze treatments and demonstrated that pathogens produce oligosaccharides and potentially other components in response to stress. Alvarez-Ordóñez and Prieto (2010) used FT-IR spectroscopy to study the ultrastructure changes of *Salmonella enterica* cells under acid, alkaline, heat, and oxidative stressed conditions. A wide variety of cellular compounds were determined to be involved in bacterial resistance to unfavorable conditions.

Conclusions

In this study, UV radiation dose in a range from 12.1 kJ/m^2 to 72.8 kJ/m^2 can inactivate *C. sakazakii* in dry infant formula ($P < 0.05$). UV dose required by FDA to inactivate bacteria in liquid food is too low for desiccation food powder. The UV radiation in combination with 55 °C and 65 °C hot water treatment decreased *D* values of *C. sakazakii* in reconstituted infant formula. FT-IR spectroscopic analyses demonstrated that UV radiation resulted in changes of DNA, structural proteins, and phospholipids of bacterial cell and this subsequently caused bacterial injury and death.

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