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Validation of radio-frequency dielectric heating system for destruction of *Cronobacter sakazakii* and *Salmonella* species in nonfat dry milk

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ABSTRACT

Cronobacter sakazakii and *Salmonella* species have been associated with human illnesses from consumption of contaminated nonfat dry milk (NDM), a key ingredient in powdered infant formula and many other foods. *Cronobacter sakazakii* and *Salmonella* spp. can survive the spray-drying process if milk is contaminated after pasteurization, and the dried product can be contaminated from environmental sources. Compared with conventional heating, radio-frequency dielectric heating (RFDH) is a faster and more uniform process for heating low-moisture foods. The objective of this study was to design an RFDH process to achieve target destruction (log reductions) of *C. sakazakii* and *Salmonella* spp. The thermal destruction (decimal reduction time; D-value) of *C. sakazakii* and *Salmonella* spp. in NDM (high-heat, HH; and low-heat, LH) was determined at 75, 80, 85, or 90°C using a thermal-death-time (TDT) disk method, and the z-values (the temperature increase required to obtain a decimal reduction of the D-value) were calculated. Time and temperature requirements to achieve specific destruction of the pathogens were calculated from the thermal destruction parameters, and the efficacy of the RFDH process was validated by heating NDM using RFDH to achieve the target temperatures and holding the product in a convection oven for the required period. Linear regression was used to determine the D-values and *z*-values. The D-values of *C. sakazakii* in HH- and LH-NDM were 24.86 and 23.0 min at 75°C, 13.75 and 7.52 min at 80°C, 8.0 and 6.03 min at 85°C, and 5.57 and 5.37 min at 90°C, respectively. The D-values of *Salmonella* spp. in HH- and LH-NDM were 23.02 and 24.94 min at 75°C, 10.45 and 12.54 min at 80°C, 8.63 and 8.68 min at 85°C, and 5.82 and 4.55 min at 90°C, respectively. The predicted and observed destruction of *C. sakazakii* and *Salmonella* spp. were in agreement, indicating that the behavior of the organisms was similar regardless of the heating system (conventional vs. RFDH). Radio-frequency dielectric heating can be used as a faster and more uniform heating method for NDM to achieve target temperatures for a postprocess lethality treatment of NDM before packaging.

Key words: radio-frequency dielectric heating, nonfat dry milk, *Cronobacter sakazakii*, *Salmonella* species

INTRODUCTION

Nonfat dry milk is a widely used dairy product that can be consumed directly or used as an ingredient in various food products, including powdered infant formula (**PIF**). Neither NDM nor PIF are sterile, and the possibility of postpasteurization contamination of milk cannot be ruled out (Olsen et al., 2004; Brooks, 2010). *Cronobacter sakazakii* and *Salmonella* spp. have been associated with several sporadic outbreaks due to contaminated NDM and PIF (Bowen and Braden, 2006; Cahill et al., 2008; Astley, 2012) and have been isolated from NDM and PIF products (Louie et al., 1993; Iversen and Forsythe, 2004; Flynn, 2011). Although *C. sakazakii* and *Salmonella* spp. cannot survive milk pasteurization (Read et al., 1968; Osaili et al., 2009), they can survive spray-drying temperatures if postpasteurization contamination of the milk occurs (LiCari and Potter, 1970; Arku et al., 2008).

Both *C. sakazakii* and *Salmonella* spp. have been classified as class A pathogens (clear evidence of causality) in PIF (FAO/WHO, 2004a,b). *Cronobacter sakazakii* can result in life-threatening infections in infants such as bacteremia, meningitis, sepsis, cerebritis, and necrotizing enterocolitis (FAO/WHO, 2004a; Fiore et al., 2008; Lehner, 2010), and *Salmonella* spp. can cause severe diarrhea in infants that can result in death (FAO/WHO, 2004a).

Radio-frequency dielectric heating (**RFDH**) is an electro-heating process in which electric energy is converted into electromagnetic waves that subsequently generate volumetric heat within a product (Rowley, 2001; Piyasena et al., 2003; Marra et al., 2009). Foods consist of dipoles (such as water) and ions (such as

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salts), which contribute to heat generation within a food product during RFDH (FDA, 2000). During RFDH treatment, a food product is placed between the capacitor plates of an RFDH unit and an alternating electric field is applied. Frictional heat is generated within the food product due to (1) dipole rotation (the dipoles in the food align themselves along the electric field and oscillate continuously along the changing electric field), and (2) ionic depolarization (the ions in the food move toward the opposite charged regions of the electric field and migrate continuously according to the changing electric field; Piyasena et al., 2003; Ramaswamy and Tang, 2008; Marra et al., 2009). Because radio frequencies can interfere with radar and communication systems, the frequencies approved by Electromagnetic Compatibility Regulations and Federal Communications Commission for industrial, scientific, and medical applications are 13.56 ± 0.00678, 27.12 ± 0.16272, and 40.68 ± 0.02034 MHz (FDA, 2000; Rowley, 2001; Marra et al., 2009). The advantages of RFDH over conventional heating are better volumetric heating, more uniform heating, greater penetration power, lower surface overheating, fewer cold spots, shorter treatment time, improved energy efficiency, and better process control (FDA, 2000; Marra et al., 2009).

Although RFDH is used in baking, drying, and defrosting, commercial applications for food pasteurization and sterilization are scarce in North America (FDA, 2000; Rowley, 2001; Ramaswamy and Tang, 2008). Research on RFDH applications for the thermal inactivation of foodborne pathogens has been explored in mashed potatoes for *Clostridium sporogenes* (Luechapattanaporn et al., 2004), scrambled eggs for *C. sporogenes* (Luechapattanaporn et al., 2005), milk for *E. coli* and *Listeria innocua* (Awuah et al., 2005), ground beef for *E. coli* (Guo et al., 2006), apple cider for *E. coli* (Geveke and Brunkhorst, 2008), and pork luncheon meat for *Bacillus cereus* and *C. perfringens* (Byrne et al., 2010). Reports on *C. sakazakii* and *Salmonella* spp. in NDM or PIF suitable for commercial application are nonexistent. Prior research on destruction of these pathogens in NDM and PIF was done either in liquid-rehydrated systems or at temperatures $\leq$70°C (Iversen at al., 2004; Kim and Park, 2007; Walsh et al., 2011). Therefore, the objective of this study was to determine the thermal processing parameters (decimal reduction time, D-value, at 75, 80, 85, and 90°C; and thermal resistance constant, z-value) of *C. sakazakii* and *Salmonella* spp. in high-heat (**HH**) and low-heat (**LH**) NDM using a water bath and thermal-death-time (**TDT**) disks; and to validate an RFDH system for decontamination of NDM. Because HH- and LH-NDM are used for different applications in various food products, both were included in this study.

MATERIALS AND METHODS

Experimental Design

A 5-strain cocktail of *C. sakazakii* or *Salmonella* spp. was used in this study because both of the organisms have been implicated in outbreaks from contaminated NDM. The D- and z-values of *C. sakazakii* or *Salmonella* spp. in HH-NDM (ConAgra Foods, Menomonie, WI) and LH-NDM (Dairy America, Fresno, CA) were first determined using custom-designed stainless steel TDT disks (6.0 cm in diameter and 0.5 cm high; University of Nebraska, Lincoln) and a hot-water bath (Precision Scientific, Chicago, IL). On the basis of results obtained from the TDT disks, the RFDH experiment was designed to validate microbial destruction. The TDT disks and RFDH studies were considered independent experiments; HH- and LH-NDM were inoculated and heat-treated randomly. Three replications were conducted for all treatments, and each microbial enumeration was done in duplicate. Linear regression graphs were plotted using SAS version 9.1 (SAS Institute Inc., Cary, NC). All comparisons for *C. sakazakii* or *Salmonella* spp. in HH- and LH-NDM at respective temperatures were conducted with Student's t-test at $P \leq 0.05$ in SAS 9.1 (SAS Institute Inc.).

Bacterial Cultures

Cronobacter sakazakii and *Salmonella* spp. strains were selected based on risk and involvement in outbreaks associated with NDM or PIF, isolated from food processing plants, and used in published research. *Cronobacter sakazakii* 29544 and BAA-894 were obtained from the American Type Culture Collection (ATCC, Manassas, VA), and 1 environmental isolate and 2 processing-plant isolates of *C. sakazakii* were obtained from Applied Food Safety Laboratory, University of Nebraska (Lincoln). *Salmonella* serovars Agona BAA-707, Tennessee 10722, and Typhimurium 13311 were obtained from ATCC, and *Salmonella* Montevideo and Senftenberg (processing plant isolates) were obtained from the Food Safety and Defense Laboratory, Kansas State University (Manhattan). All strain designations were confirmed using API 20E (bioMérieux, Durham, NC).

Inoculum Preparation and Inoculation Procedure

All organisms were individually grown on tryptic soy agar (**TSA**; Difco, Becton, Dickinson Co., Sparks, MD), and isolated colonies were maintained at 4°C on TSA plates. An individual colony of each strain was transferred from TSA to 10 mL of tryptic soy broth