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 heat-

ing 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* (Luechapattanaorn et al., 2004), scrambled eggs for *C. sporogenes* (Luechapattanaorn 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 $\leq 70^\circ\text{C}$ (Iversen et 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

Figure 1. Thermal-death-time disk (a) containing inoculated NDM and connected to a thermocouple; and (b) schematic. Color version available in the online PDF.

(Difco) and incubated at 37°C for 24 h. A loop (25 µL) of each strain was individually transferred into 40 mL of tryptic soy broth in centrifuge tubes and incubated at 37°C for 24 h. Strains were then centrifuged at $5,520 \times g$ and -4°C for 15 min, the supernatant was discarded, and pellets were reconstituted in 40 mL of 0.1% peptone water (Difco). Five strains of *C. sakazakii* or *Salmonella* spp. were mixed and transferred into separate, sanitized spray bottles (300 mL).

High-heat or LH-NDM in sanitized plastic tubs (9.4 L, Rubbermaid, Atlanta, GA) was inoculated with a 5-strain cocktail of *C. sakazakii* or *Salmonella* spp. by spraying 1 squirt (~0.8 mL) per 10 g of NDM. Inoculated NDM was dried with open lids at 37°C for 24 h (to achieve the original moisture content of NDM; data not presented), and then ground using a food processor (Ninja, Euro-Pro Operating LLC, Boston, MA) for 1 min to homogenize the inoculum.

Heat Treatment

TDT Disks. The TDT disks (Figure 1) used in this study were similar to those used by Jin et al. (2008); however, the disks in this study were manufactured from stainless steel instead of aluminum. Eight grams of inoculated NDM was placed in a sanitized TDT disk, sealed, and transferred to a hot-water bath (preset at 75, 80, 85, or 90°C). Temperatures of samples (in TDT disks) and the hot-water bath were monitored using

thermocouples (Hypodermic Needle Probes, Omega Engineering Inc., Stamford, CT) and a data logger (Measurement Computing USB-TC and MCC DAQ Software, Norton, MA). Once the target temperatures were reached, samples were held for 0 to 80 min and then transferred from the hot-water bath to a cold-water bath (~4°C) at specific time intervals. The processed samples were cooled to ~10°C and immediately enumerated for *C. sakazakii* or *Salmonella* spp.

RFDH. Inoculated HH-NDM (5 g) or LH-NDM (7 g) was placed in a sanitized circular container (Aqua Lab, Decagon, Pullman, WA), covered with a lid, and then wrapped in Parafilm M (Pechiney Plastic Packaging, Chicago, IL). The sealed, inoculated container was placed at the center of the circular high-density polyethylene tray (0.3 cm thick, 22.4 cm in diameter, and 2.1 cm high; Dynalab Corp., Rochester, NY) along with a noninoculated sample container (Figure 2a) to monitor the temperature using a fiber-optic probe (T1, Neoptix Inc., Québec, Canada) and a data logger (Reflex, Neoptix Inc.). The circular tray was then filled with noninoculated HH- or LH-NDM, and the product surface in the tray was leveled (with a 30-cm ruler) to a height of 2.1 cm (Figure 2b). Samples were heated in the RFDH unit (Figure 3; Strayfield Limited, Theale, UK) set at a frequency of 27.12 MHz, power of 3 kW, and electrode distance of 120 mm until target temperatures were achieved. Samples were then transferred to a convection oven (Thelco, GCA/Precision Scientific, Schaar

Figure 2. Inoculated and noninoculated (connected to fiber-optic probe) NDM in sealed containers in high-density polyethylene tray for radio-frequency dielectric heating: (a) before covering with noninoculated NDM; and (b) after covering with noninoculated NDM. Color version available in the online PDF.

Scientific Company, Chicago, IL) pre-set at the designated temperatures for specific time periods. Processed samples were then transferred to a cold-water ($\sim 4^{\circ}\text{C}$) bath. The samples were cooled to $\sim 10^{\circ}\text{C}$ and immediately enumerated for *C. sakazakii* or *Salmonella* spp.

Microbial Enumeration

The treated product (5 or 7 g) was transferred to a stomacher bag to which 45 or 43 mL of peptone water (0.1%) was added. The sample was mixed by stomacher (Smasher, AES Laboratoire, Bruz, France) for 1 min, and serial dilutions (1:10) were subsequently prepared using peptone water. The samples were spread-plated on TSA and incubated at 37°C for 24 h. Noninoculated HH- and LH-NDM were also plated on TSA to determine the background microbial load and the presence of *C. sakazakii* and *Salmonella* spp.

Determination of D- and z-Values

Regression lines were generated from the log of viable microbes versus time, and D-values were calculated as absolute values of inverse of the slopes. For z-values, regression lines were generated from the log of D-values versus temperatures, and z-values were calculated as absolute values of inverses of the slopes (Al-Holy et al., 2009).

Observed Versus Predicted Microbial Destruction

On the basis of thermal destruction parameters (obtained from TDT disk study), the times required to

achieve 3-log reduction (predicted log reduction) of *C. sakazakii* and *Salmonella* spp. in HH- and LH-NDM were calculated. The RFDH study was then conducted to validate the log reduction (observed log reduction) of *C. sakazakii* and *Salmonella* spp. in HH- and LH-NDM at respective temperatures for calculated times. The observed reductions of *C. sakazakii* and *Salmonella* spp. were plotted against predicted reductions and compared.

RESULTS AND DISCUSSION

Mean times required for NDM to achieve the target temperatures in the TDT disks and RFDH unit from an initial temperature of $\sim 27^{\circ}\text{C}$ are presented in Table 1. For all the temperatures, the time required to achieve the target temperatures was less ($P \leq 0.05$) in the RFDH unit compared with TDT disks (Table 1). These results confirmed that RFDH can heat a low-moisture food product faster than conventional heating methods. In the TDT disks, heat was transferred mainly by conduction between TDT disks and NDM, whereas in the RFDH unit, heat was generated within NDM by dielectric heating.

The initial total plate counts in HH- and LH-NDM were 2.05 ± 0.05 and 2.41 ± 0.06 log cfu/g ($n = 3$), respectively (which were within the standards; < 4 log cfu/g; USDEC, 2010), and the samples were negative for *C. sakazakii* and *Salmonella* spp. (detection limit of 1 log cfu/g). The thermal inactivation of *C. sakazakii* and *Salmonella* spp. in HH- and LH-NDM using TDT disks is presented in Figures 4 and 5. The TDT curves

Table 1. Time (min) required to achieve the target temperatures for NDM in thermal-death-time (TDT) disks and radio-frequency dielectric heating (RFDH) unit¹

Heating method	Temperature (°C)			
	75	80	85	90
TDT disks	7.94 ± 0.96 ^a	9.51 ± 1.11 ^a	8.86 ± 0.72 ^a	10.72 ± 0.87 ^a
RFDH	4.26 ± 0.23 ^b	4.54 ± 0.32 ^b	4.95 ± 0.30 ^b	5.50 ± 0.38 ^b

^{a,b}Values within a column differ significantly ($P \leq 0.05$).¹Mean ± SE (n = 3).

(log of D-value vs. temperature) were used to calculate z -values (Figure 6).

The initial counts of *C. sakazakii* or *Salmonella* spp. in inoculated NDM were ~6.0 log cfu/g (data not shown). The D-values of *C. sakazakii* or *Salmonella* spp. for HH- and LH-NDM at the same temperatures were similar ($P > 0.05$), except for *C. sakazakii* in HH- and LH-NDM at 80°C (Table 2). The D-values of *C. sakazakii* and *Salmonella* spp. varied from 23.00 to 26.25 min at 75°C, 7.52 to 13.75 min at 80°C, 6.03 to 8.68 min at 85°C, and 3.05 to 5.82 min at 90°C, respectively (Table 2). The z -values of *C. sakazakii* in HH- and LH-NDM were 23.77 and 26.28°C, respectively, whereas z -values of *Salmonella* spp. in HH- and LH-NDM were 26.92 and 20.92°C, respectively (Table 2).

Observed log reductions were in agreement with predicted log reductions (Figure 7), except that observed log reductions of *C. sakazakii* in HH- and LH-NDM at 90°C and *Salmonella* spp. in HH-NDM at 90°C were >1.5 log cfu/g compared with predicted reductions. Overall, the predicted and observed log reductions for *C. sakazakii* and *Salmonella* spp. were similar using TDT disks and RFDH methods, indicating that destruction of organisms is mainly due to the thermal effect. The RFDH reduced the time to achieve the target temperatures compared with conventional heating. Geveke et al. (2007), however, reported that RFDH treatment of pulp-free orange juice at 21.1 kHz and 60°C reduced the *Escherichia coli* K12 population by 2.1 log cfu/mL, whereas the *E. coli* population did not decrease when

Figure 3. Radio-frequency dielectric heating unit. Color version available in the online PDF.

Figure 4. Thermal inactivation of *Cronobacter sakazakii* using thermal-death-time disks at 75, 80, 85, and 90°C in high-heat (HH) and low-heat (LH) NDM. Error bars indicate SE. Color version available in the online PDF.

juice was heated at 60°C using the conventional method, suggesting that nonthermal effects could contribute to these differences. Geveke and Brunkhorst (2008) reported that the *E. coli* K12 population in apple cider was reduced by 4.8 log cfu/mL by RFDH at 60°C and 21 kHz compared with no reduction using conventional heating at 60°C. Ukuku et al. (2012) suggested that nonthermal effects of RFDH on microbial cells could be due to the decrease in the microbial cell surface hydrophobicity and loss of negative ions.

Thermal inactivation data of *C. sakazakii* and *Salmonella* spp. in NDM or foods with low water activity (at the temperatures investigated in this study) are not available in the literature. The published thermal destruction parameters for *C. sakazakii* and *Salmonella* spp. in various dry products were derived from experiments conducted either at temperatures $\leq 70^\circ\text{C}$ or as rehydrated products (such as infant formula). Lound et al. (2011) reported that D-values of non-acid-adapted *Salmonella* Typhimurium in powdered egg albumin heat-treated in a hot water bath were 99.56, 43.90, and 15.54 min at 72, 77, and 82°C, respectively. These D-values were greater than the D-values for *Salmonella* spp. in NDM at 75, 80, and 85°C in the current study. Perhaps the differences in *Salmonella* spp. serovars or strains used or inoculation procedures [dried at 50°C

after spray inoculation before heat treatment by Lound et al. (2011) vs. dried at 37°C after spray inoculation in this study] could have contributed to the differences in D-values. In addition, compositional (e.g., proteins, carbohydrates, fat) differences between egg albumin and NDM could contribute to these differences in the D-values of *Salmonella* spp. VanCauwenberge et al. (1981) reported variability in D-values at 49°C for *Salmonella* spp. (8 different serotypes) in corn flour, which ranged from 18 to 594 min.

Luechapattaporn et al. (2004) reported that D-values of *Clostridium sporogenes* (PA3679) in mashed potatoes (water activity = 0.998), using TDT tubes submerged in a heated oil bath, ranged from 2.16 to 0.61 min at 115.6 to 121.1°C. From this data, they calculated F_0 values (time required to destroy specific microorganisms in specified medium at 121.1°C), which validated the microbial destruction using RFDH. Luechapattaporn et al. (2005) used the same procedure to validate D-values with RFDH but using scrambled eggs as the medium; moreover, both studies of Luechapattaporn et al. (2004, 2005) maintained the samples in an RFDH unit submerged in a water chamber to reduce the fringe (or edge) effect during the entire process. In our study, samples were transferred to a

Figure 5. Thermal inactivation of *Salmonella* spp. using thermal-death-time disks at 75, 80, 85, and 90°C in high-heat (HH) and low-heat (LH) NDM. Error bars indicate SE. Color version available in the online PDF.

Table 2. The D-values (decimal reduction time; min) and z-values (temperature increase required to obtain a decimal reduction of the D-value; °C) of *Cronobacter sakazakii* and *Salmonella* spp. in high-heat (HH) and low-heat (LH) NDM heated in thermal-death-time disks¹

Item	<i>C. sakazakii</i>		<i>Salmonella</i> spp.	
	HH-NDM	LH-NDM	HH-NDM	LH-NDM
Temperature (°C)				
75	24.86 ± 2.33	23.00 ± 3.17	23.02 ± 1.53	24.94 ± 3.50
80	13.75 ± 1.22 ^a	7.52 ± 0.48 ^b	10.45 ± 0.15	12.54 ± 0.98
85	8.00 ± 0.93	6.03 ± 0.24	8.63 ± 1.04	8.68 ± 2.61
90	5.57 ± 0.79	5.37 ± 1.24	5.82 ± 1.18	4.55 ± 0.33
z-value	23.77 ± 3.70	26.28 ± 4.73	26.92 ± 3.65	20.92 ± 0.40

^{a,b}Values within a row and under respective species differ significantly ($P \leq 0.05$).

¹Mean ± SE (n = 3).

convection oven for process time after attaining the target temperatures in RFDH. Mashed potatoes and scrambled egg obviously had greater moisture contents than NDM (<5%), plus the microorganisms differed. Products with greater moisture contents have greater dielectric constants and loss factors, which result in greater conversion of radio frequencies into thermal energy compared with products with low moisture contents (Komarov et al., 2005).

Chen et al. (2013) studied the functional properties of HH- and LH-NDM by heating NDM in a RFDH unit to 75, 80, 85, and 90°C and holding the samples in a convection oven up to 125.67, 57.75, 25.0, and 11.50 min, respectively. They reported that whey protein nitrogen indices (WPNI) of heat-treated HH-NDM samples were similar to that of nonheated control (1.48 mg/g) and ranged from 1.32 to 1.48 mg/g; however, WPNI of HH-NDM heated at 80°C for 57.75 min and at 85°C for 10 and 25 min (1.32 to 1.34 mg/g) were lower than that of nonheated control. Chen et al. (2013) also reported that the WPNI of LH-NDM heated at

temperatures ≥80°C (5.78 to 6.48 mg/g) were lower than that of nonheated control (7.24 mg/g). Although WPNI of some NDM differed ($P \leq 0.05$) from the nonheated controls, all samples maintained their classification standards (>6 mg/g for HH-NDM, and <1.5 mg/g for LH-NDM), except for LH-NDM heated at 85°C for 25 min. Nitrogen solubility indices (NSI) of all

Figure 6. Log D-value (decimal reduction time) versus temperature for *Cronobacter sakazakii* and *Salmonella* spp. in high-heat (HH) and low-heat (LH) NDM using thermal-death-time disks. Error bars indicate SE. Color version available in the online PDF.

Figure 7. Predicted versus observed log reductions of (a) *Cronobacter sakazakii*, and (b) *Salmonella* spp. in high-heat (HH) and low-heat (LH) NDM. RFDH = radio-frequency dielectric heating. Color version available in the online PDF.

heated NDM at temperatures $\geq 80^{\circ}\text{C}$ were lower (89.0 to 87.9% and 88.3 to 89.4% for HH- and LH-NDM, respectively; $P \leq 0.05$) than that of nonheated controls (90.6 and 93.3% for HH- and LH-NDM, respectively). Chen et al. (2013) also reported that color of HH-NDM was not affected by heat treatment, except that HH-NDM heated at 90°C for 0 min (yellow-blue color, $b^* = 13.27$) was yellower than that of nonheated control ($b^* = 11.46$). Low heat-NDM was also not affected by the heat treatment, except that LH-NDM heated at 75 and 85°C for 25 and 10 min, respectively (red-green color, $a^* = -2.51$ and -2.44 , respectively) were greener than nonheated control ($a^* = -1.89$), and LH-NDM heated at 90°C for 0 min ($b^* = 14.43$) was yellower than nonheated control ($b^* = 12.27$).

CONCLUSIONS

Treatment by RFDH can be used to heat NDM, thus shortening the overall postprocess lethality treatment before packaging, and to minimize food safety risk. The effects of RFDH on the functional properties of NDM, such as WPNI, solubility, color, and flowability, should also be evaluated. Thermal resistance of *C. sakazakii* and *Salmonella* spp. in NDM should be further validated under commercial conditions.

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