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Superheated steam effectively inactivates diverse microbial targets despite mediating effects from food matrices in bench-scale assessments

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ABSTRACT

Sanitation in dry food processing environments is challenging due to the exclusion of water. Superheated steam (SHS) is a novel sanitation technique that utilizes high temperature steam to inactivate microorganisms. The high sensible heat of SHS prevents condensation on surfaces. Here we evaluated SHS thermal inactivation of various vegetative and spore forming bacteria and fungi and determined the effect of food matrix composition on SHS efficacy. Capillary tubes with vegetative cells (*Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, or *Enterococcus faecium*), *Aspergillus fischeri* ascospores, or *B. cereus* spores (100 μL) were SHS treated at 135 ± 1 °C for 1 or 2 s. After 1 s, SHS achieved a reduction of 10.91 ± 0.63 $\log_{10}$ CFU/mL for vegetative cells, 2.09 ± 0.58 $\log_{10}$ ascospores/mL for *A. fischeri*, and 0.21 ± 0.10 $\log_{10}$ spores/mL for *B. cereus*. SHS treatment achieved significant reductions in vegetative cells and fungal ascospores ($p < 0.05$), however *B. cereus* spores were not significantly reduced after 2 s and were determined to be the most resistant of the cell types evaluated. Consequently, peanut butter compositions (peanut powder, oil, and water) and milk powder (whole and nonfat) inoculated with *B. cereus* spores on aluminum foil coupons (2 × 3 × 0.5 cm) were tested. The $D_{161°C}$ values for *B. cereus* spores ranged from 46.53 ± 4.48 s (6 % fat, 55 % moisture, a_w: 0.927) to 79.21 ± 14.87 s (43 % fat, 10 % moisture, a_w: 0.771) for various peanut butter compositions. Whole milk powder had higher $D_{161°C}$ (34.38 ± 20.90 s) than nonfat milk powder (24.73 ± 6.78 s). SHS (135 ± 1 °C) rapidly (1 s) inactivated most common vegetative bacterial cells; however *B. cereus* spores were more heat resistant. *B. cereus* spore inactivation was significantly affected by product composition ($p < 0.05$). Compared to the log-linear model (R^2 0.81–0.97), the Weibull model had better fit (R^2 0.94–0.99). Finally, the ease of peanut butter removal from surfaces increased while the ease of non-fat dry milk removal decreased with the increasing SHS treatment duration. However, allergen residues were detectable on surfaces regardless of SHS treatment. The findings from this study can inform the development of pilot-scale research on SHS.

1. Introduction

The challenge of ensuring food safety in low moisture food (LMF) has been recognized for more than a decade (Beuchat et al., 2013; Gurtler et al., 2014; Podolak et al., 2017; Rana et al., 2021; Syamaladevi et al., 2016b). More recently, the importance of surface sanitation to prevent cross-contamination in LMF processing environments has been identified because water is not generally used in these environments (Jackson et al., 2008; Zhang et al., 2019). Although aqueous sanitation is well studied and effective (Burnett and Hagberg, 2014; Cai et al., 2020; Frank and Chmielewski, 1997; Mercer and Somers, 1957), the introduction of water into manufacturing spaces which are otherwise dry has been shown to increase microbial harborage (FDA, 2018). Most conventional dry sanitation activities (brushing, scraping, vacuuming) rely simply on physical removal (cleaning) which has known limitations in achieving the multifaceted goals of sanitation (He et al., 2022). For example, physical removal strategies alone are not strongly antimicrobial (Grasso et al., 2015). Nor are these methods effective in allergen removal (Chen et al., 2022; Jackson et al., 2008). And methods using hand tools like brushes, scrapers, or rags cannot penetrate recessed niches in food processing equipment when compared to fluid or gaseous treatments. This leaves many gaps regarding clean-breaks and the effective control of food safety hazards. Superheated steam (SHS) represents a novel technology that can improve sanitation efforts in LMF processing

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environments.

SHS, sometimes referred to as "dry steam," offers many advantages as a dry sanitation technology (Ban et al., 2014; Kwon et al., 2019; Park et al., 2021). SHS has greater thermal energy than an equivalent amount of water at a given temperature (James et al., 1998). SHS can effectively penetrate cavities, crevices, and follicles that may provide protection for microbial targets (Morgan et al., 1996a). It is a non-polluting, non-chemical technology with low energy consumption (Morgan et al., 1996b). It works by heating saturated steam at increasingly high temperatures (125 °C to >300 °C) while maintaining the same pressure (van Deventer and Heijmans, 2001). The ability of SHS to dry materials is due to the addition of sensible heat that raises the material's temperature above the corresponding saturation temperature at a given pressure (100 °C at atmospheric pressure). Unlike saturated steam, a temperature decrease will not result in condensation of the SHS as long as the temperature is still greater than the saturation temperature at the given processing pressure (Pronyk et al., 2004).

Because of these attributes, SHS may be an effective tool for sanitization in LMF processing environments. Sanitation in food manufacturing environments includes both cleaning and sanitization, each with different goals (Park et al., 2021). The purpose of cleaning is to remove bulk food material and allergenic residues. Without effective cleaning, remaining residues can cross-protect microbes during sanitization, create harborage points for microbes, leading to allergen cross-contact. The purpose of sanitization is to inactivate any remaining microbes (Rovira, 2016). Sanitization must address a range of spoilage and pathogenic biota – both fungal and bacterial – which include many diverse microbial targets with different degrees of sensitivity to the sanitizer's mechanism of action (Cai and Snyder, 2022; Djukic et al., 2016; Marriott et al., 2006). Without effective sanitization, food plants face an increased risk of microbial cross-contamination. Sanitization efficacy can be mediated by the presence of residual food soils which interfere with the thermal or chemical inactivation of pathogens or spoilage microbes (Cai et al., 2018). These issues have been well established across many sanitization technologies, but we hypothesize that the high temperature of SHS may overcome these limitations. The goal of this study was to: 1) determine microbial sensitivity to SHS among diverse targets (*Salmonella enterica*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus*, *Enterococcus faecium*, *Aspergillus fischeri*), 2) evaluate the mediating effect of food residues on thermal inactivation of *B. cereus* spores, and 3) investigate the effect of SHS treatments on food residue removal from surfaces.

2. Material and methods

2.1. Strains and inoculum preparation

The microbial strains used in this study are presented in Table 1. For vegetative cells (*Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, *Enterococcus faecium*), a loopful of frozen stock for each strain was initially inoculated into Brain Heart Infusion (BHI) broth (BD, Thermo Fisher Scientific, Waltham, MA, USA) and incubated at 35 ± 2 °C for 24 ± 3 h. Broth suspension was streaked onto BHI agar plates and incubated at 35 ± 2 °C for 24 ± 3 h. An isolated colony was transferred from stock plates into BHI broth followed by incubation at 35 ± 2 °C for 20 ± 3 h. After incubation, the culture broth was centrifuged at 10,000 RPM (19,900 ×*g*) for 5 min (Eppendorf 5804R, Eppendorf, NY, USA), and the cell pellet was washed twice in 0.1 % phosphate buffered saline (PBS) (BD, Thermo Fisher Scientific, Waltham, MA, USA). After washing, the cell pellets were resuspended in 10 % volume of the initial inoculum carrier (0.1 % PBS), to achieve a high cell concentration of ~11.0 $\log_{10}$ CFU/mL. A cell cocktail for each microorganism was prepared by mixing an equal volume of inoculum from each strain.

For *B. cereus* spores, the frozen stock of each strain mentioned in Table 1 was initially inoculated into BHI broth and incubated at 35 ± 2 °C for 24 ± 3 h. Broth suspension was streaked onto BHI agar plates and incubated at 35 ± 2 °C for 24 ± 3 h. An isolated colony was transferred from stock plates into BHI broth followed by incubation at 35 ± 2 °C for 24 h. From 24 h culture, 100 μL inoculated broth suspension was spread onto AK #2 agar (BD, Thermo Fisher Scientific, Waltham, MA, USA) and incubated for 5 days at 35 ± 2 °C. After 5 days of incubation, the presence of refractile spores was confirmed using a phase-contrast microscope (AMScope, Irvine, CA, USA). After confirmation, the plate was flooded with 8 mL of 4 °C sterile dH_2O, and growth was scraped off the agar using a sterile spreader (VWR, Wayne, PA, USA), collected into a 50 mL Eppendorf tube (Stellar Scientific LLC, Berkeley, CA, USA), and centrifuged at 10,000 RPM for 5 min (Eppendorf 5804R, Eppendorf, NY, USA). The pellet was resuspended in 3.5 mL dH_2O and washed three times. After the final wash of the spore preparation, the pellet was resuspended in 5 mL of 4 °C 50 % *v*/v ethanol (Fisher Scientific, Waltham, MA, USA), and incubated at 4 °C in a tube rotator (Cole-Parmer, Vernon Hills, IL, USA) running at 15 RPM for 12 h to eliminate vegetative bacterial cells. After 12 h, the Eppendorf tube was centrifuged at 10,000 RPM (19,900 ×*g*) for 5 min and the spore pellet was resuspended in 3.5 mL dH_2O and washed three times. The final spore pellet was resuspended in 10 % volume of the initial inoculum carrier (dH_2O), to achieve a high spore concentration of 8.20 ± 0.21 $\log_{10}$ spores/mL. Cocktails were prepared by mixing equal volumes from each strain.

For fungi, the frozen stock of *A. fischeri* was streaked onto Potato Dextrose Agar (PDA) (BD, Thermo Fisher Scientific, Waltham, MA, USA) acidified to pH 3.5 using sterile 10 % tartaric acid (BD, Thermo Fisher Scientific, Waltham, MA, USA), and incubated at 25 °C for 30 days. After 30 days of incubation, the presence of ascospores was confirmed using a phase-contrast microscope (AmScope, Irvine, CA, USA). After confirmation, the plate was flooded with 8 mL of 4 °C sterile dH_2O, and growth was scraped off the agar using a sterile spreader (VWR, Wayne, PA, USA), collected into a 50 mL Eppendorf tube (Stellar Scientific LLC,

Table 1
Microbial targets used in thermal inactivation trials.

Organism	Strain	Source	Reference
Salmonella enterica	Tennessee 2053H	Thyme	Blessington et al., 2012; Peña-Meléndez et al., 2011; Rana et al., 2021
	Elmsbuettel 1236H	Peanut butter	
	PT-30	Almonds	
Escherichia coli	32-DB	Green pepper	Rana et al., 2021
	32-C	Green pepper	
	Meat-1	Meat	
Listeria monocytogenes	F6-0665	Cheese	den Bakker et al., 2010; Cai et al., 2020; Sauders et al., 2004
	R2-0574	Cheese	
	M2-0018	Cheese	
Bacillus cereus	FSL A1-0001	Freeze dried milk	Kent et al., 2016
	FSL K6-0995	Milk powder	
	FSL K6-2822	Milk powder	
Enterococcus faecium	NRRL B-2354	Dairy utensils	Kopit et al., 2014
Aspergillus fischeri		Heat processed fruit products	Buerman et al., 2019; Buerman et al., 2021

Berkeley, CA, USA), and centrifuged at 10,000 RPM (19,900 ×*g*) for 5 min (Eppendorf 5804R, Eppendorf, NY, USA). Cell pellets were washed twice in 0.1 % PBS. After washing, the cell pellet was resuspended in 10 % volume of the initial inoculum carrier (0.1 % PBS). Prior to SHS treatment the fungal suspension was heat treated at 75 °C for 5 min to inactivate any vegetative cells or asexual spores. A fungal ascospore concentration of 5.93 ± 0.25 $\log_{10}$ spores/mL was recorded after initial heat treatment.

2.2. SHS system

A schematic diagram of the SHS equipment (HGA-S, MHI Inc., Cincinnati, OH) and its components are shown in Fig. 1a. A type-K, 0.5 mm diameter thermocouple (OMEGA Engineering, Norwalk, CT, USA) and an 8 channel handheld data logger (OMEGA Engineering, Norwalk, CT, USA) were used to record the chamber temperature before SHS treatment with a time interval of 2 s. All treatments in the SHS study were performed at a distance of 4 cm (Fig. 1a) from the SHS inlet based on the preliminary temperature distribution experiment data.

2.3. SHS inactivation of microbial cells contained in capillary tubes

Concentrated cell suspension (20 μL) was injected into glass micro-hematocrit capillary tubes (inner diameter, outer diameter × length: 1.5, 1.8 × 40 mm) (Kimble chase, Vineland, NJ, USA) using Luer lock syringes with attached needles (Air-Tite, Virginia Beach, VA, USA). A repeating syringe dispenser (Pb600-1, Hamilton, Reno, NV, USA) was used to ensure uniform injection volumes. Initial population in the capillary tubes for each organism is reported in Table 3. After injection, the capillary tubes were flame sealed (to prevent test sample leakage and evaporation) and 5 × 20 μL capillary tubes (100 μL total volume) were used in each experiment. Injected capillary tubes were stored at room temperature (21 ± 1 °C) before SHS treatment. The five capillary tubes were held in a custom holder (length × width × depth: 38 × 12.7 × 5 mm) 4 cm from the SHS inlet and treated at 135 ± 1 °C for 1 or 2 s in the bench-scale SHS chamber (Fig. 1b). Treatment temperature of 135 ± 1 °C was used to represent the low spectrum of SHS temperatures. A K-type thermocouple was placed at the center of the sample holder and at the geometric center of the capillary tube surface during SHS treatment at 135 ± 1 °C (Fig. 2).

After SHS treatment at 135 ± 1 °C, the capillary tubes were transferred to an ice ethanol bath (70 % EtOH) for 5 min to stop further thermal inactivation due to residual heat. Total time was recorded. This include treatment time, come-up time, as well as the time to transfer to the ice ethanol bath, but outcomes were grouped based on exposure time (e.g., 1 or 2 s). Treatment chamber was equilibrated to 135 ± 1 °C between different capillary tube experiments. The tubes were then removed from the bath and any remaining ethanol was removed from their surfaces using a sterilized paper towel. The tubes were then transferred to double-layered sample bags (Whirl-Pak™, Madison, WI, USA) and crushed using a rubber mallet (Workpro®, Trenton, NJ, USA) to release the cell suspension. Sample dilutions were performed using 0.1 % PBS and appropriate dilutions were plated either on BHI agar (bacteria) or PDA (fungi) plates and incubated at 35 ± 2 °C and 25 ± 2 °C, for 24 h and 5 days, respectively. SHS treatment was performed on three biological replicates.

2.4. Food matrix compositions and inoculation

Instant nonfat dry milk (NFDM) (Nestle Carnation, Switzerland), whole milk powder (WMP) (Nestle Nido fortified, Switzerland), peanut powder (Great value, Walmart, Bentonville, AR, USA), and peanut oil (Great value, Walmart, Bentonville, AR, USA) were purchased from a national retailer. Six different peanut butter compositions were prepared in the lab using varying levels of peanut powder, peanut oil, and sterile dH_2O as described in Table 2 and stomaching (Stomacher® 400 Lab Blender Series, Seward, UK) for 10 min until fully homogenized as previously described (He et al., 2011).

One day before the SHS treatment, 9.9 g of test matrix (peanut butter, NFDM, or WMP) was spot inoculated with 10 spots of 10 μL concentrated *B. cereus* spore cocktail in sterile sample bags (Whirl-Pak™, Madison, WI, USA). Inoculated samples were hand massaged for 30 s and equilibrated for 24 h at room temperature in a biosafety cabinet (Thermo Fisher Scientific, Waltham, MA, USA). Following equilibration samples were stomached for 10 min. The initial population in the inoculated samples after 24 h equilibration ranged from 6.2 to 7.5 $\log_{10}$

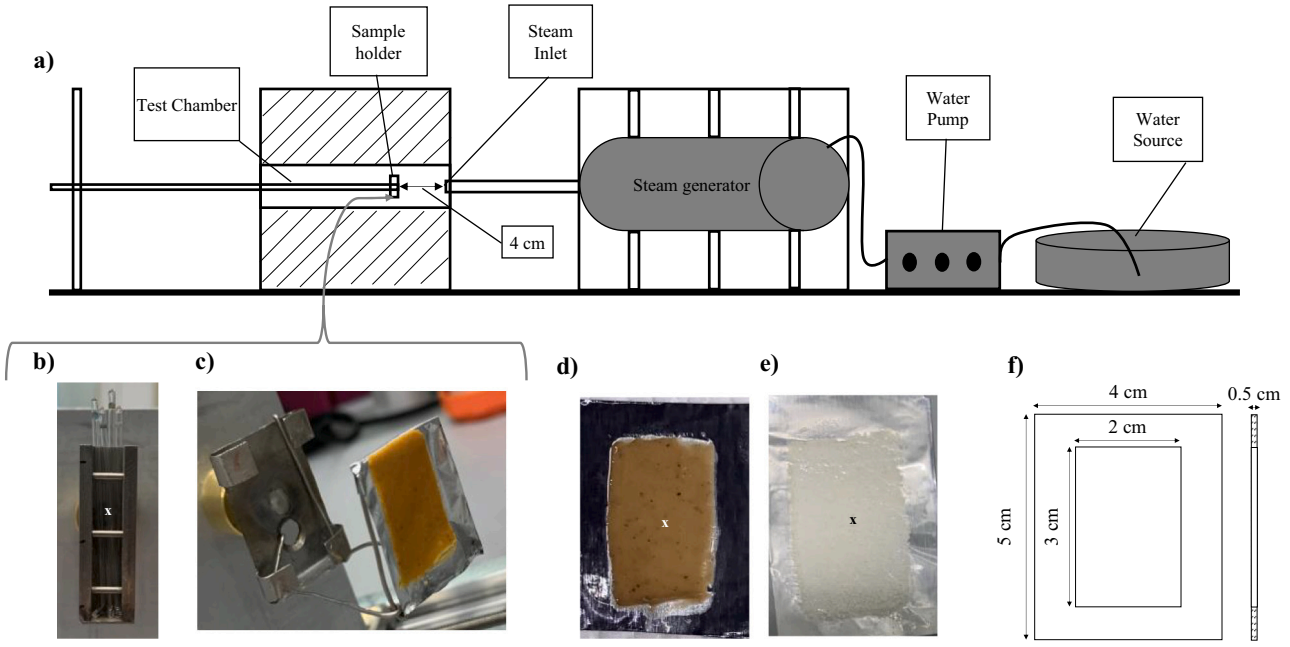

Fig. 1. (a) Schematic diagram of SHS generation equipment, (b) capillary tube treatment stage, (c) aluminum foil treatment stage, (d) peanut butter sample placement on aluminum foil, (e) NFDM sample placement on aluminum foil, and (f) the dimensions of custom plastic mold (left: top view, right: sectional front view). "*x*" represents thermocouple location at the geometric center.

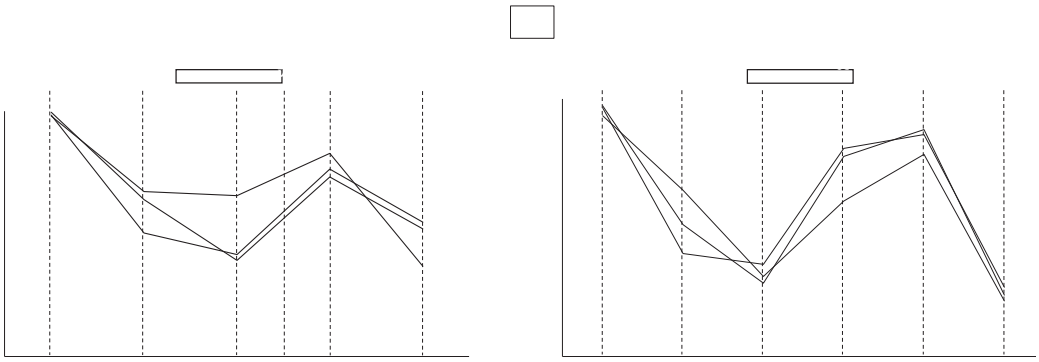

Fig. 2. Geometric center of the capillary tube surface temperature profile during (a) 1 s, (b) 2 s exposure at 135 ± 1 °C SHS treatment. The three lines represent three different experimental replicates.

Table 2
Weight (g) of each component in peanut butter composition and the corresponding water activity (a_w).

Composition	Peanut powder (g)	Peanut oil (g)	Water (g)	a_w
1	30	20	0	0.235 ± 0.062
2	30	20	5	0.771 ± 0.018
3	30	15	0	0.228 ± 0.025
4	30	25	25	0.960 ± 0.001
5	30	10	15	0.927 ± 0.025
6	30	0	35	0.970 ± 0.001

spores/g.

2.5. SHS inactivation of microbial cells in food matrix on foil surfaces

The preparation procedure developed by Park et al. (2021) was adapted. Test coupons (length × width: 2.5 × 3.5 cm) were made of aluminum foil (Reynolds Wrap®, Auckland, NZ) and sterilized in an autoclave (Steris®, Mentor, OH, USA) at 121 °C for 30 min under 15 psi of pressure. A 0.5 mm thin-film of peanut butter, NFDM, or WMP inoculated with *B. cereus* spores was cast on sterile aluminum foil coupons using a custom plastic mold (length × width × thickness: 2 × 3 × 0.5 cm) (Fig. 1f). By sliding a scraper (Warner Manufacturing Co, Plymouth, MN) over the mold, the excess test sample was scraped away, resulting in a uniform test sample layer. The initial population of *B. cereus* spores was 8.22 ± 0.67, 7.70 ± 0.27, and 8.40 ± 0.33 $\log_{10}$ spores/g for peanut butter, NFDM, and WMP, respectively. The placement of the test sample on foil coupons is shown in Fig. 1d and e. Sterile dH_2O was brushed on aluminum coupons prior to casting NFDM or WMP samples to assist in sample adhesion. Coupons with NFDM or WMP were then air-dried for 5 h at room temperature in a biosafety cabinet prior to SHS treatment. Following air drying, the water activity (a_w) of the NFDM or WMP samples was measured at room temperature using a dew point water activity meter (Aqualab Series 4 TE, Decagon Devices Inc., Pullman, WA, USA). Additionally, the a_w of the food samples was measured after SHS treatment (161 ± 1 °C for 30 s) using a dew point water activity meter after food sample equilibration to room temperature. Temperature across the test coupon varied up to 2.20 ± 0.19 °C from the geometric center.

The sample-coated coupon was then attached to a custom coupon holder as shown in Fig. 1c. A type-K, 0.5 mm diameter thermocouple was used to monitor the temperature of the test sample at the geometric center (Fig. 1d and e) using an 8 channel handheld data logger set to a time interval of 2 s. Mounted coupons were then treated at 161 ± 1 °C in the bench-scale SHS chamber, 4 cm from the SHS inlet. Treatment temperature of 161 ± 1 °C was used to prevent condensation of vapor on food matrix as reported by Park et al., 2021 for treatment temperatures below 150 °C. After treatment, the coupons were quickly transferred to standard sample bags containing 1.0 mL of 0.1 % PBS, and the bags were placed in an ice bath for 5 min to stop the thermal process. After cooling, the bags were hand massaged for 60 s until no clumps were observed. Sample dilutions were performed using 0.1 % PBS and appropriate dilutions were plated on BHI agar and incubated at 35 ± 2 °C prior to enumeration of colony forming units (CFU). Since 0.30 ± 0.03 g (peanut butter composition) or 0.20 ± 0.02 g (NFDM or WMP) of the deposited samples were homogenized in 1 mL of PBS before plating 100 μL on BHI plates, the minimum detection limit in this study was 33 CFU/g for peanut butter compositions and 50 CFU/g for NFDM or WMP. Treatment was performed on three biological replicates.

2.6. Modeling SHS inactivation kinetics

Thermal destruction curves were constructed by plotting the $\log_{10}$ survivor counts vs the treatment time. Inactivation kinetics of *B. cereus* spores in different food matrix compositions were estimated by fitting the log-linear model and the Weibull model to the experimental data using Geeraerd and Van Impe inactivation model fitting tool (Geeraerd et al., 2005) in MS excel (2021–22, Microsoft, WA).

The log-linear (first-order kinetic) model used to estimate inactivation kinetics was as follows:

$$log\left(\frac{N}{N_0}\right) = -\frac{t}{D_T} \quad (1)$$

where N and N_0 are *B. cereus* spore populations (CFU/g) at times t and surviving spore population after achieving target temperature at CUT, respectively, t (s) is the treatment time at the target temperature, and D_T is the time (s) required to reduce the spore population by 10-fold at a specified temperature T (°C) and a_w.

The Weibull model used to estimate inactivation kinetics was as follows:

$$logN = logNo - \left(\frac{t}{\delta}\right)^p \quad (2)$$

where N and N_0 are *B. cereus* spore counts (spores/g) at times t and surviving spore population after achieving the target temperature at CUT, respectively, t (s) is the treatment time at the target temperature,

and δ and p are the scale and shape parameters of Weibull inactivation curve, where $p > 1$ indicates a concave downward trend, meaning the rate of microbial inactivation increases over time, and $p < 1$ indicates a concave upward trend of the inactivation curve, meaning the rate of microbial inactivation decreases overtime, and $p = 1$ indicates a linear trend (Cullen et al., 2009; Peleg and Cole, 1998).

2.7. Cleaning treatment

A 0.5 mm thin-film layer of peanut butter (J.M. Smucker co, Orrville, OH, USA) or a dusting of NFDM (Nestle Carnation, Switzerland) was cast on sterile aluminum foil coupons using the custom plastic mold (Fig. 1f). Unlike peanut butter, NFDM was not deposited in a 0.5 mm thin-layer due to lack of sample adhesion required for SHS treatment. Coupons dusted with NFDM were transferred to a desiccator (Pyrex, Greencastle, PA, USA) equilibrated at 100 % RH (using 100 % dH_2O) and room temperature for 24 h to increase the adhesion between NFDM and aluminum foil (Chen et al., 2022). After 24 h, NFDM coupons were air-dried for 5 h at room temperature in a biosafety cabinet to remove any additional moisture. The sample-coated coupon was then treated at 161 ± 1 °C for 30, 60, or 300 s in the bench-scale SHS chamber. After SHS treatment, coupons were subjected to cleaning with a hygienic scraper (Warner Manufacturing Co, Plymouth, MN) for three consecutive passes. Before scraping and following each pass of the scraper, the mass of the remaining food soil was measured by an analytical balance (Aczet Pvt. Ltd., Mumbai, India) with an accuracy of 0.1 mg. An image of each coupon was also recorded after each pass of the scraper. Additionally, following the third pass of the scraper, Lateral Flow Devices (LFD) (3 M, St. Paul, MN) were used to detect milk or peanut allergenic residues.

2.8. Scanning electron microscopy of NFDM microstructure before and after SHS treatment

NFDM and WMP were deposited on stainless steel coupons and transferred to a desiccator equilibrated at 100 % RH and room temperature for 24 h to increase the adhesion between milk powder and coupon. After 24 h, NFDM coupons were air-dried for 5 h in a biosafety cabinet and then treated at 161 ± 1 °C for 60 s in the bench-scale SHS chamber. Non-treated and treated samples were coated with Au/Pd for 30 s using a vacuum desk (Denton Vacuum Desk V, Moorestown, NJ, US). Following Au/Pd coating the coupons were mounted on a standard 12-mount sample holder and imaged using an LEO 1550 FE scanning electron microscope (Keck, Zeiss, Jena, Germany) at the Cornell Center for Materials Research.

2.9. Statistical analysis

Microbial inactivation before and after SHS treatment for capillary tube experiments was tested for significance by an analysis of variance (ANOVA) model using the aov function in R-studio (Chambers et al., 1992). The results of SHS inactivation for different food matrix compositions, treatment time, and their interaction were examined for statistical significance by two-way ANOVA. Microbial counts were log-transformed to better align with the model assumptions of normality and homogeneous variance. The R-studio computer program (version 1.3.959, 2009–2020 RStudio, PBC) was used for all calculations.

3. Results and discussion

3.1. Vegetative cells and fungal ascospores were significantly reduced by short, dynamic SHS exposure, while B. cereus spores were not significantly reduced

In commercial application of SHS as a manually applied surface treatment, short, dynamic treatment exposures are anticipated. Therefore, we initially evaluated the degree of inactivation achieved under 1 and 2 s SHS exposures across a panel of pathogenic and spoilage biota. Dynamic temperature profiles for three experimental replicates were recorded for both 1 s (Fig. 2a) and 2 s (Fig. 2b) treatment durations. Temperature across the length of the capillary tubes varied up to 1.5 ± 0.2 °C from the surface geometric center. Within 1 s of exposure, the surface temperature of the capillaries increased to 128–130 °C. The capillary tube surface temperature was dynamic throughout the course of this brief exposure and never reached the treatment chamber temperature (135 °C), a come-up time (CUT) of 30 ± 5 s was necessary to achieve temperature equilibration. Thermocouples inserted into the center of the internal cavity of a filled capillary tube revealed that temperatures reached 99 ± 0.3 °C within 1 s of treatment, likely because the aqueous matrix will not exceed 100 °C under atmospheric pressure prior to a phase change to saturated steam (Caupin, 2005; Sanz et al., 2004). The treatment conditions highlighted in Fig. 2 were used to: 1) assess the degree of inactivation achieved under short, dynamic temperature exposures and 2) compare the thermoresistance among relevant microbial targets using the 1 and 2 s dynamic profiles.

The results of thermal inactivation across a panel of pathogenic and spoilage biota shown in Table 3 were used to identify the most resilient target for subsequent food matrix studies (Mazzotta, 2001; Murphy et al., 2004; Smith et al., 2001; Smelt and Brul, 2014). Table 3 describes the treatment time (1 or 2 s exposure) as well as the total time (including

Table 3
Thermal inactivation of different microorganisms under SHS at 135 ± 1 °C.

Organism	Treatment time (s)	Total time (s)[a]	Survivors (Log_{10}CFU/mL)
E. coli	0	0	11.0 ± 0.17
	1	5	ND
	1	5	ND
	1	5	ND
	2	6	ND
	2	6	ND
	2	7	ND
Salmonella	0	0	11.31 ± 0.11
	1	5	ND
	1	5	ND
	1	5	ND
	2	6	ND
	2	6	ND
	2	6	ND
E. faecium	0	0	9.99 ± 0.45
	1	6	1.48
	1	5	ND
	1	5	ND
	2	7	ND
	2	6	ND
	2	6	ND
L. monocytogenes	0	0	11.35 ± 0.09
	1	5	ND
	1	7	ND
	1	5	ND
	2	6	ND
	2	7	ND
	2	7	ND
B. cereus (spores)	0	0	8.29 ± 0.14
	1	6	8.11
	1	7	8.16
	1	7	7.97
	2	8	7.74
	2	8	7.65
	2	8	7.69
Aspergillus fischeri	0	0	5.93 ± 0.25
	1	5	3.23
	1	6	4.39
	1	5	3.89
	2	7	ND
	2	6	ND
	2	7	ND

[a] Total time include treatment time, sample loading-unloading, and transfer to ice bath.

treatment time, sample loading-unloading, and transfer to ice bath) for each biological replicate. A 1 s SHS exposure at 135 ± 1 °C reduced vegetative cells by 9.20 ± 1.10 $\log_{10}$ CFU/mL (*Enterococcus faecium*) to 11.35 ± 0.09 $\log_{10}$ CFU/mL (*L. monocytogenes*). Compared to bacterial vegetative cells, fungal ascospores were more resistant to heat treatment. A 1 s SHS exposure at 135 ± 1 °C resulted in a reduction of 2.09 ± 0.58 $\log_{10}$ ascospores/mL *Aspergillus fischeri* while increasing treatments to 2 s led to 5.93 ± 0.25 $\log_{10}$ ascospores/mL reduction. Overall, *B. cereus* spores were most heat resistant under the tested treatment conditions. For *B. cereus* spores, SHS treatment at 135 ± 1 °C for 1 s achieved a reduction of 0.21 ± 0.10 $\log_{10}$ spores/mL, and 2 s of SHS treatment led to a 0.60 ± 0.04 $\log_{10}$ spores/mL reduction.

Results of an ANOVA showed that during 1 and 2 s SHS treatment, bacterial vegetative cells and fungal ascospores were significantly reduced ($p < 0.05$); however, *B. cereus* spores were not significantly reduced under these conditions. The enhanced resistance of *B. cereus* spores compared to fungal ascospores may be attributable to the difference in function between these cell types. While bacterial spores are resilient cell forms that promote survival, fungal ascospores are reproductive structures (Snyder et al., 2019). During *B. cereus* sporulation, a low-water protective cortex consisting of a peptidoglycan and keratin is formed. The low-water content of the spore core is crucial to its heat resistance during thermal treatment (Beaman and Gerhardt, 1986; Smelt and Brul, 2014). Among the microbiota tested in this study, *B. cereus* spores were most heat resistant and, consequently, were selected for further isothermal studies evaluating the effect of matrix composition.

3.2. SHS inactivation of *B. cereus* spores was significantly affected by the composition of peanut butter

SHS efficacy can be impacted by the protective effect of residual food soils on a surface. In this study, we evaluated the mediating effects of peanut butter and milk powder on microbial inactivation. These matrixes represent LMFs that have been associated with pathogen contamination, resulting in recent outbreaks and recalls, and are compositionally distinct from one another. We evaluated the effect of various peanut butter compositions that ranged in fat (6–48 %), protein (19–33 %), carbohydrate (13–22 %), moisture (1–55 %), and a_w (0.228–0.970) content on the degree of *B. cereus* spore inactivation achieved during SHS treatment (Table 4). The a_w level of the matrix increased by <0.1 unit following SHS treatments (161 ± 1 °C). These dynamics are typical of SHS surface applications and, consequently, *D*-values were reported only as a function of temperature (Park et al., 2021).

The $D_{161°C}$ values ranged from a high of 79.21 ± 14.87 s (43 % fat, 10 % moisture, a_w: 0.771) to a low of 46.53 ± 4.48 s (Table 4) (6 % fat, 55 % moisture, a_w: 0.970). Two-way ANOVA results show that peanut butter composition, treatment time, and their interaction had a significant effect on thermal inactivation of *B. cereus* spores ($p < 0.05$). Individual pairwise comparisons among different compositions reveal the impacts of individual constituents (fat, moisture, protein). For example, the same 1 % moisture content was used in peanut butter composition 3 and 1; however, the fat content increased from 42 % to 48 % between these two matrixes, which resulted in a modest increase of the a_w from 0.228 ± 0.025 to 0.235 ± 0.062. Consequently, the $D_{161°C}$ value changed slightly from 52.21 ± 29.09 s to 57.30 ± 8.95 s, respectively. By contrast, peanut butter compositions 3 and 2 possessed approximately the same level of fat (42 % vs 43 %) but had a moisture content level that differed from 1 % to 10 %, which increased the a_w from 0.228 ± 0.025 to 0.771 ± 0.018. This resulted in an increase in the $D_{161°C}$ value from 52.21 ± 29.09 s to 79.21 ± 14.87 s, respectively, which may be attributed to the interaction effect with other matrix components.

Trends regarding the impact of matrix composition on thermal inactivation observed in this study are generally aligned with prior studies conducted at temperatures <100 °C. He et al. (2011) tested *Salmonella enterica* inactivation in different peanut butter compositions and reported that the $D_{90°C}$ value for the matrix composition with 50 % fat was 2.33 min while *Salmonella enterica* was not detected in the composition with 6.25 % fat following the same treatment. Similarly, Jin et al. (2018) reported the $D_{75°C}$ value at 0.5 a_w as 35.34 min (6 % fat, 27.5 % protein) and 37.7 min (27.5 % fat, 6 % protein). Finally, Gaillard et al. (1998) tested *B. cereus* spore inactivation in citrate buffer at different a_w levels and found the $D_{105°C}$ value increased to 48.48 s from 6.30 s when a_w level decreased to 0.86 from 0.98. These results are comparable to the current study where $D_{161°C}$ increased to 79.21 ± 14.87 s from 46.53 ± 4.486 s when a_w level decreased to 0.771 ± 0.018 from 0.970 ± 0.001. While the trends in compositional impacts on inactivation kinetics were sustained across these studies, the absolute results varied based on the high treatment temperature achieved by SHS.

The inactivation curves for *B. cereus* spores in different peanut butter compositions are shown in Fig. 3. The come up time (CUT) was 30 ± 2 s and the degree of microbial inactivation that occurred during the CUT was food matrix dependent. For example, peanut butter compositions with moisture content >25 % (composition 4, 5, and 6) had high spore inactivation during the CUT. At the conclusion of the CUT, initial populations were reduced to 3.32 ± 0.28 $\log_{10}$ spores/g (36 % fat, 32 % moisture, a_w: 0.960), 5.43 ± 0.19 $\log_{10}$ spores/g (6 % fat, 55 % moisture, a_w: 0.970), and 6.02 ± 0.12 $\log_{10}$ spores/g (25 % fat, 28 % moisture, a_w: 0.927). The most rapid inactivation occurred in composition 4 where 5.52 ± 0.91 $\log_{10}$ spores/g were inactivated during the CUT (Fig. 3d). Due to low survivor counts at time 0, fitting a thermal inactivation model was not possible for peanut butter composition 4 and only observed values were reported to illustrate the low survivor count (Fig. 3d). A similar phenomenon was observed in Fig. 3f where rapid

Table 4
Estimated parameters of the log-linear regression model and Weibull model for the inactivation kinetics of *B. cereus* spores in various peanut butter compositions treated by SHS at 161 ± 1 °C.

Composition	Sample composition (*w*/w)				Log linear model				Weibull model			
	Fat (%)	Protein (%)	Carbohydrate (%)	Moisture (%)	a_w	$D_{161°C}$ (s)	RMSE	R^2	δ (s)	p	RMSE	R^2
1	48	30	20	1	0.235 ± 0.062	57.30 ± 8.95	0.30	0.973	88.25 ± 10.54	1.38 ± 0.14	0.17	0.992
2	43	27	18	10	0.771 ± 0.018	79.21 ± 14.87	0.30	0.950	102.14 ± 29.71	1.25 ± 0.35	0.33	0.941
3	42	33	22	1	0.228 ± 0.025	52.21 ± 29.09	0.78	0.808	121.24 ± 6.99	2.61 ± 0.26	0.14	0.994
4[a]	36	19	13	32	0.960 ± 0.001	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5	25	27	18	28	0.927 ± 0.025	63.90 ± 8.53	0.23	0.972	44.83 ± 14.81	0.78 ± 0.15	0.22	0.974
6	6	23	15	55	0.970 ± 0.001	46.53 ± 4.48	0.57	0.858	10.35 ± 1.28	0.46 ± 0.02	0.05	0.999

[a] Thermal inactivation kinetics were not estimated for peanut butter composition 4 due to low survivor population at time 0.

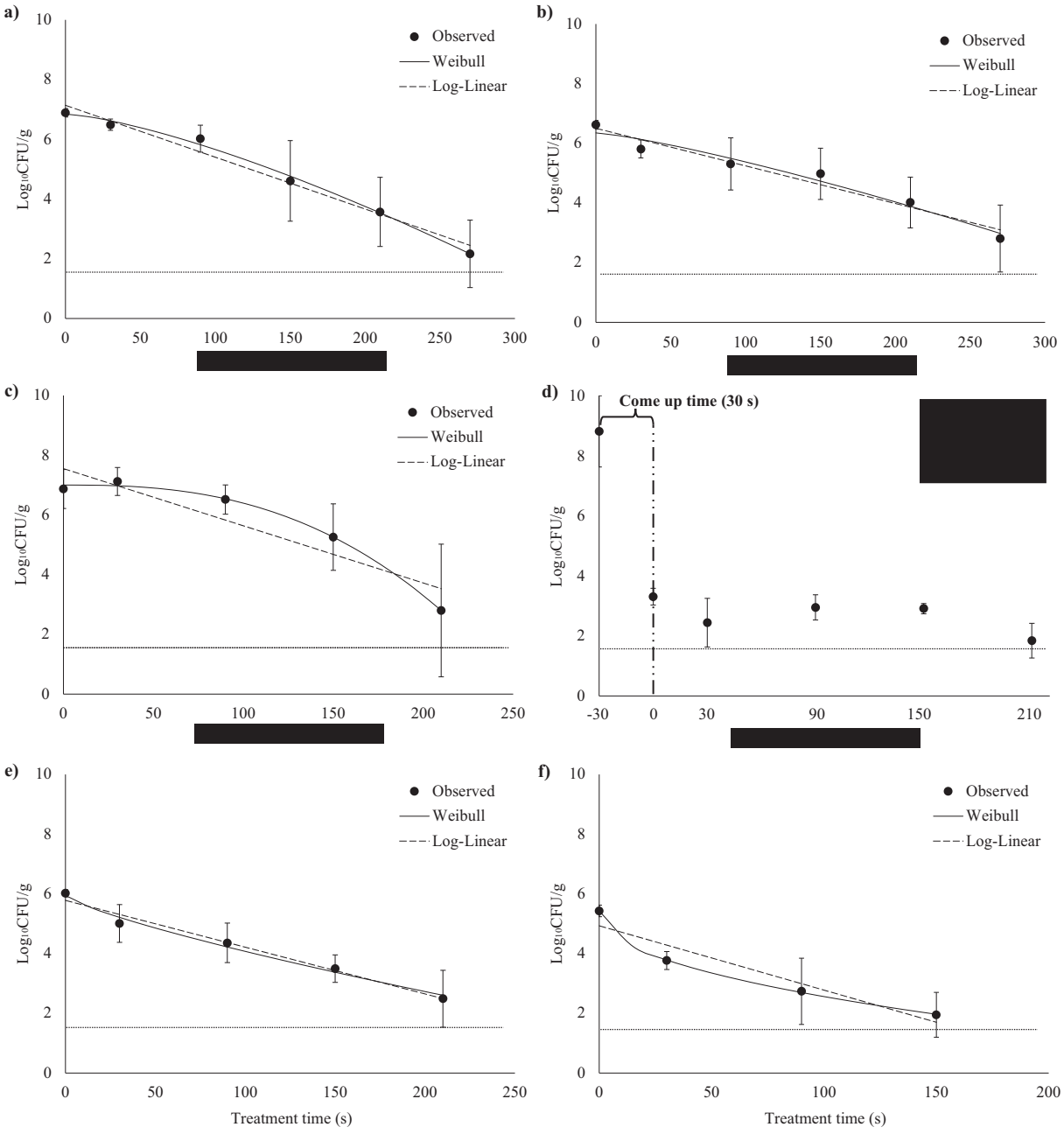

Fig. 3. Inactivation kinetics of *B. cereus* spores in peanut butter compositions: a) 1, b) 2, c) 3, d) 4, e) 5, and f) 6 during SHS treatment at 161 ± 1 °C. Compositions are described in Table 3. The dashed line represents the limit of detection at 1.50 $\log_{10}$ CFU/g. The come-up time was included in panel (d) to illustrate the rapid inactivation in this matrix.

inactivation in the CUT occurred; however, it was still possible to fit a thermal inactivation model to the survival data following the CUT in this instance.

The inactivation data were fitted with both log-linear and Weibull models. Model-fitness was comparatively better for the Weibull model as the R^2 values were higher and RMSE values were lower when compared to log-linear models (Table 4). For example, in peanut butter composition 1, the R^2 value increased to 0.992 (Weibull) from 0.973 (log-linear), and RMSE value decreased to 0.17 (Weibull) from 0.30 (log-linear). The shape parameter of the Weibull inactivation curve (p) also represents inactivation differences among peanut butter compositions (Table 4). Peanut butter compositions 1, 2, and 3, each with >40 % fat, had $p > 1$ and a concave, downward trend, meaning that the rate of microbial inactivation increased over treatment time (Fig. 3) (Cullen et al., 2009; Peleg and Cole, 1998; Chen et al., 2021). This suggests that as the SHS exposure time increased in these compositions, the remaining cells became increasingly susceptible to heat as a result of accumulated damage (Peleg and Cole, 1998; Jiang and Murthy, 2011; van Boekel, 2002). On the other hand, peanut butter compositions 5 and 6, each with >25 % moisture, had $p < 1$ had a concave, upward trend, meaning the rate of microbial inactivation decreased over time (Cullen et al., 2009; Peleg and Cole, 1998). This suggests that the most sensitive cells are inactivated first, followed by the *B. cereus* spores which are relatively more resistant (Bowman and Shenton, 2001; Chen et al., 2021; Peleg and Cole, 1998; Jiang and Murthy, 2011; van Boekel, 2002). Collectively, these results suggest the need to assess the mediating effects of specific food residues on SHS surface sanitization.

3.3. SHS inactivation of B. cereus spores was faster in NFDM than in WMP

We additionally assessed the effect of two commercially available milk powder compositions that ranged in fat (0 and 30 %), protein (23 and 35 %), carbohydrate (37 and 52 %), moisture (8 and 9 %), and a_w (0.165 and 0.185) content on degree of *B. cereus* spore inactivation by SHS (Table 5). Spore forming bacteria are of particular concern for manufacturers of powdered dairy ingredients due to their ability to withstand harsh environmental and processing conditions such as spray drying and biofilm formation (Alvarenga et al., 2018a; Alvarenga et al., 2018b; Gopal et al., 2015; Tatsinkou Fossi et al., 2017). This is particularly true for milk powders sold on international markets where spore count is an important quality criterion. Because of the resistance of spores to many lethality treatments, processors often rely on preventing the introduction of spores into raw milk (Miller et al., 2015). The major difference between the milk powders tested in the current study was the level of fat resulting in a decrease in the $D_{161°C}$ to 24.73 ± 6.78 s from WMP was 34.38 ± 20.90 s (Table 5). The protective effect of WMP was also observed by Wei et al. (2020), reporting that for *Salmonella enterica* inactivation in milk powder, the $D_{85°C}$ (a_w 0.20) = 370.20 s and was higher for WMP compared to that of NFDM (339.0 s). Similarly, Sekhon et al. (2021) tested *Salmonella enterica* inactivation in NFDM and WMP and also found that $D_{85°C}$ = 594.0 s was higher for WMP compared to for NFDM (552.0 s) (Sekhon et al., 2021). The present study differs from this previous work based on microbial target and treatment temperature. Most previous studies have focused on the inactivation of vegetative cells, rather than spore formers. However, spore counts represent a quality metric important to international sales of milk powder. Because SHS treatments are >100 °C, spore inactivation is feasible.

An appreciable increase in a_w levels was recorded following SHS treatments (161 ± 1 °C). The a_w level increased from 0.185 to 0.489 for NFDM and 0.165 to 0.505 for WMP. This was due to initial moisture condensation during SHS treatment at 161 ± 1 °C and the high temperature of lactose crystallization, which results in release of water molecules (Baechler et al., 2005). Additional studies indicated that increasing the SHS temperature reduced this initial moisture condensation during CUT. Two-way ANOVA test results showed that the inactivation rate of *B. cereus* spores in milk powders was significantly impacted by matrix type, treatment time, and their interaction ($p < 0.05$). The inactivation curves for *B. cereus* in NFDM and WMP are shown in Fig. 4. Notably, at more severe treatment conditions, a combination of results both above and below the limit of detection (no colonies) was obtained among the three replicates. In cases where results were below the detection limit, the detection limit of 1.51 $\log_{10}$ CFU/g was identified as the result. However, all replicates were below the detection limit, those results were not included in the thermal inactivation model parameter determination.

SHS at 161 °C reduced survivor counts to the approximate limit of detection (1.70 $\log_{10}$ CFU/g) within 300 s for both NFDM and WMP (Fig. 4). CUT was recorded at 30 ± 2 s, and heat treatment during CUT lead to initial spore inactivation of 0.5 ± 0.3 $\log_{10}$ spores/g (WMP) and 0.8 ± 0.1 $\log_{10}$ spores/g (NFDM). The inactivation data were fitted with both a log-linear and Weibull model. The dynamic nature of the SHS treatment resulted in somewhat larger standard deviations (Figs. 3 and 4). While this can impact the derived survival parameters, these results can be used to generate a range of values inclusive of that variation. Model-fitness was comparatively better for the Weibull model as the R^2 values were higher and RMSE values were lower than for log-linear models (Table 5). The shape parameter of the Weibull inactivation curves for both NFDM and WMP were $p > 1$, indicating a concave downward trend and implying that the rate of microbial inactivation increased over time (Cullen et al., 2009; Peleg and Cole, 1998). This may suggest that in milk powder, as the SHS exposure time increases, *B. cereus* spores have increased susceptibility due to accumulated damage (Peleg and Cole, 1998; Jiang and Murthy, 2011; van Boekel, 2002). Interestingly, a recent study by Kim et al. (2020) found that inactivation of *B. cereus* spores on stainless steel yielded <2 log reduction after 5 min of exposure at temperatures >200 °C. However, this study specified only the target temperature and did not indicate the temperature achieved by the surface, the come-up time, or the distribution of the temperature across the surface. These experimental difference likely account for the differences in inactivation rates observed in our study.

3.4. The ease of food residue removal from surfaces following SHS treatment was dependent on both food matrix composition and SHS treatment duration

SHS is a sanitization technology and does not replace cleaning steps to remove food residues from surfaces. However, if cleaning steps are not completely successful and some residual food remains on surfaces, the high temperature of SHS treatment may increase food residue adhesion to equipment and make future cleaning and sanitation more difficult. Therefore, we assessed the changes in the ease of food residue removal from aluminum foil following SHS treatment for a single composition of each matrix, peanut butter (50 % fat, 18 % carbohydrate) and NFDM (0 % fat, 52 % carbohydrate), which represented distinct differences in fat and carbohydrate levels. Following SHS treatment, the weight of residual peanut butter or NFDM was recorded following each pass of a cleaning tool (Fig. 5) to quantify the difficulty of residue removal using conventional cleaning methods (Chen et al., 2022).

Results show that the ease of food residue removal from surfaces following SHS treatment (161 ± 1 °C) was dependent on both food matrix and SHS treatment duration. Peanut butter was readily removed following SHS treatment as 99.07 ± 0.15 % of the residue weight on the surface was removed (Fig. 5a). However, only 36.22 ± 2.88 % of the NFDM was able to be removed following SHS treatment (Fig. 5b). This aligns with results from previous studies that have shown that hygienic scraping is more effective for removing pastes from surfaces as opposed to powders (Chen et al., 2022; Moerman and Mager, 2016). The ease of peanut butter removal from the surface became easier with increasing SHS treatment duration (Fig. 5a). Residual peanut butter left on the surface after the 3rd pass of the scraper was 0.23 ± 0.24 %, 0.16 ± 0.24 % and 0.03 ± 0.48 % respectively, following a 30 s, 60 s, or 300 s SHS treatment. On the contrary, the ease of NFDM removal from the surface became more difficult with increasing SHS treatment duration. Residual NFDM left on surfaces after the 3rd pass of the scraping tool was 15.33 ± 8.70 %, 19.38 ± 4.16 % and 28.42 ± 0.21 %, respectively, for 30 s, 60

Table 5
Estimated parameters of the log-linear regression model and Weibull model for the inactivation kinetics of *B. cereus* spores in NFDM and WMP treated by SHS at 161 ± 1 °C.

Sample	Sample composition				Log linear model				Weibull model			
	Fat (%)	Protein (%)	Carbohydrate (%)	Moisture (%)	a_w	$D_{161°C}$ (s)	RMSE	R^2	δ (s)	p	RMSE	R^2
NFDM	0	35	52	9	0.185 ± 0.013	24.73 ± 6.78	0.46	0.912	47.24 ± 5.80	1.80 ± 0.26	0.24	0.976
WMP	30	23	37	8	0.165 ± 0.005	34.38 ± 20.90	0.38	0.905	58.49 ± 10.83	1.71 ± 0.41	0.33	0.928

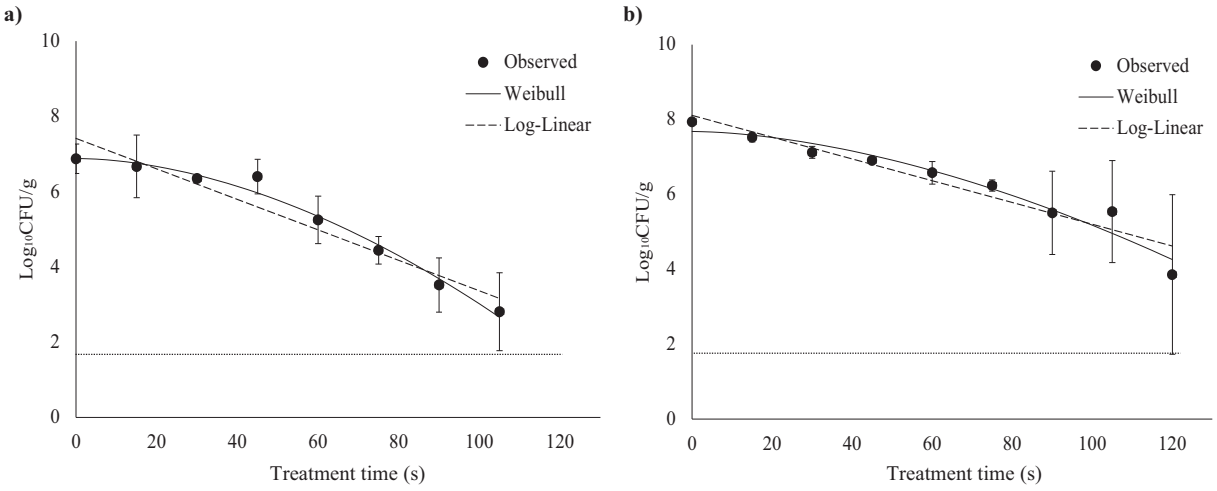

Fig. 4. Inactivation kinetics of *B. cereus* spores in a) NFDM and b) WMP during SHS treatment at 161 ± 1 °C. The dashed line represents the limit of detection at 1.7 $\log_{10}$ CFU/g.

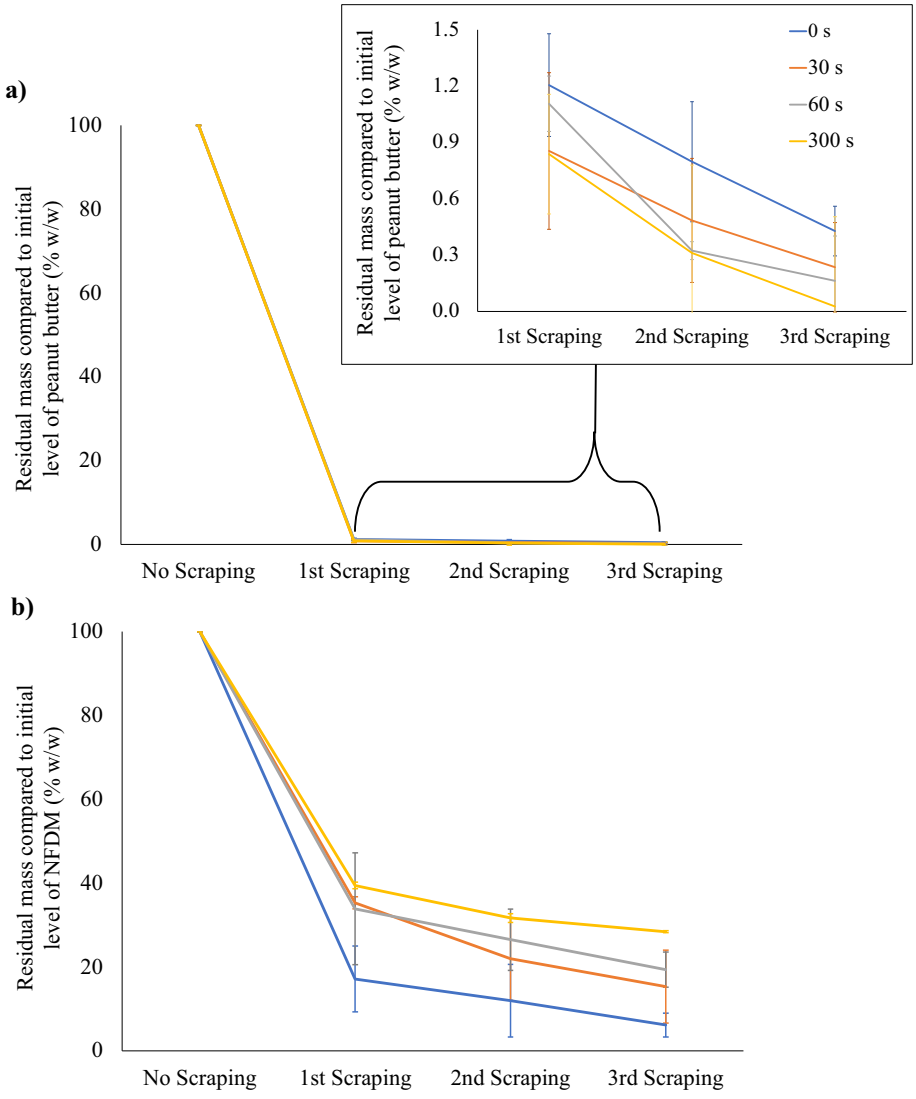

Fig. 5. The residual mass following consecutive passes of a hygienic scraper for a) peanut butter and b) NFDM treated by SHS at 161 ± 1 °C for 0 s, 30 s, 60 s, or 300 s.

s, or 300 s SHS treatment. NFDM contains 52 % carbohydrate (primarily lactose) and at SHS temperatures, treatment times increased the stickiness of NFDM on the coupon surface. These findings emphasize the importance of effective and complete cleaning (i.e. food residue removal) prior to application of SHS, particularly in some food systems where the composition of the product results in high surface adhesion under SHS treatments. In the absence of effective initial cleaning, SHS will result in "burn-on" making subsequent cleaning and sanitation cycles increasingly difficult.

Another goal of cleaning and sanitation is allergen removal. Allergens are proteins and high temperature SHS may impact the ability of commercial tests used in sanitation monitoring to detect those proteins. Therefore, we assessed the effect of SHS treatment on the results from specific allergen residue LFDs. The peanut proteins were consistently detected on aluminum foil by peanut LFDs for both untreated and SHS treated (161 ± 1 °C) peanut butter contaminated surfaces (Supplementary Fig. S1b). Increasing SHS treatment duration (300 s) did not reduce peanut allergen detection. Similarly, the milk protein LFD test reported "overload" results for both untreated and SHS treated NFDM (Supplementary Fig. S2b). Currently, many dry sanitation methods are not effective "clean break" interventions that sufficiently reduce the risk of allergen residue cross-contact and pathogen cross-contamination. While our bench-scale findings suggest that SHS may be an effective antimicrobial agent, these results do not suggest improved allergen residue management. Visibly, peanut butter was more easily removed than NFDM after SHS treatment (Supplementary Fig. S1a, Supplementary Fig. S2a). Only a slight change in color was observed for peanut butter after 60 s of treatment. However, a severe color changes were recorded for NFDM after SHS treatment (Supplementary Fig. 1a, Supplementary Fig. S2a). SEM images showing the microstructure changes in NFDM and WMP particles on SS coupons before and after the SHS treatment of 161 ± 1 °C for 60 s are presented in Fig. 6. SEM images were not generated for peanut butter because the composition of peanut butter is incompatible with the SEM sample preparation method.

The composition of milk powder, notably the high lactose content, impacted ease of removal and microstructural changes following SHS treatment. In spray-dried milk powder, proteins, fat, and air pockets are dispersed in metastable amorphous lactose which is formed as a result of rapid evaporation of water molecules (Saito, 1985; Vuataz, 2002). Dispersion of untreated NFDM and WMP samples was observed in Fig. 6a and c. Exposing milk powder to high temperatures induces crystallization in this metastable amorphous lactose (Saito, 1985; Vuataz, 2002). Fig. 6b and d show similar crystallization in both NFDM and WMP (Fig. 6d) and migration of fat molecules from the interior of the milk particle (Baechler et al., 2005; Fäldt and Bergenståhl, 1996; Saito, 1985; Wursch et al., 1984). This phenomenon was evident in WMP where SHS treatment led to separation of the fat molecules (Fig. 6d). Generally, changes due to lactose under SHS treatment decreased the ease of removal of milk powders from surfaces.

4. Conclusion

This study quantitatively investigated SHS as a novel sanitation tool for LMF processing environments. Results from first objective indicate that SHS (135 ± 1 °C) rapidly (1 s) inactivated most common vegetative bacterial cells (*Salmonella enterica*, *E. coli*, *Listeria monocytogenes*, and *Enterococcus faecium*); however, fungal ascospores and, to a greater extent, *B. cereus* spores were more heat resistant. This suggests that in industrial applications, SHS may be an effective sanitation tool where short exposure times are anticipated for the elimination of vegetative pathogens. Results from the inactivation of heat resistant *B. cereus* spores were significantly impacted by food matrix composition in both peanut butter and milk powders. Inactivation of *B. cereus* spores embedded in food product represents a worst-case scenario (e.g. no surface cleaning, targeting bacterial spores rather than vegetative pathogens) wherein inactivation required longer exposure times and was not instantaneous. Finally, the composition of the food affected food residue removal (i.e. cleaning) following SHS treatments. The high fat peanut butter was

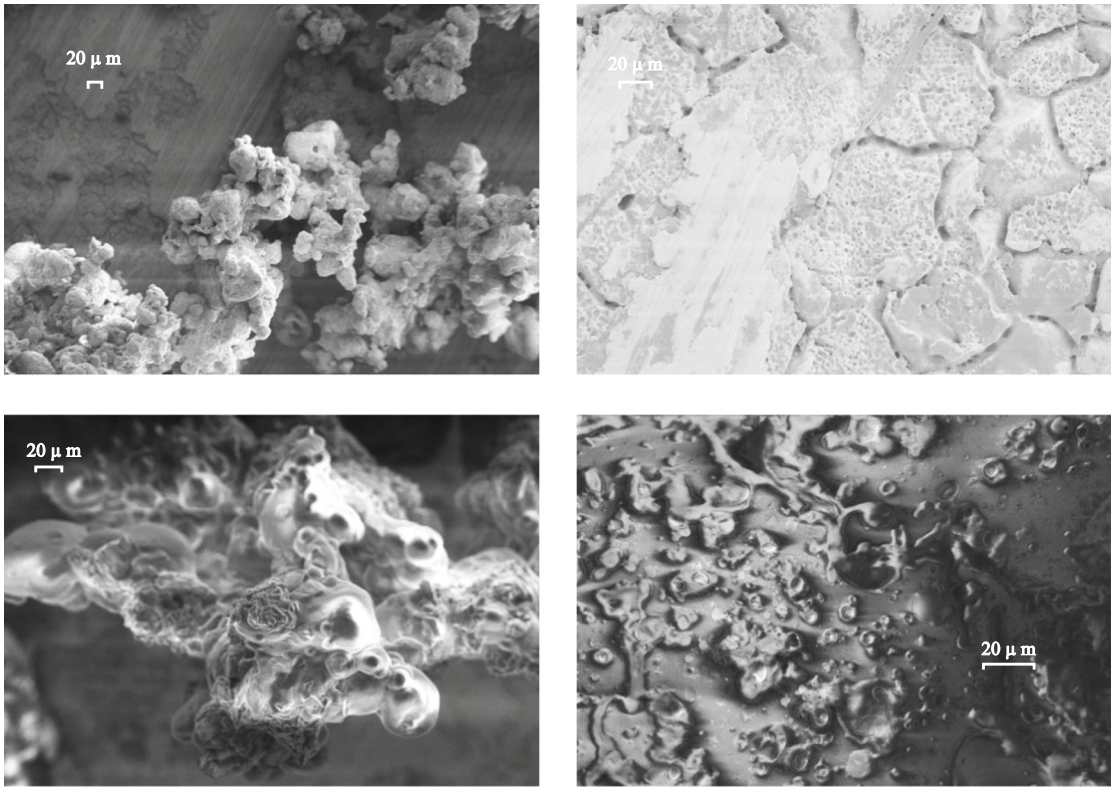

Fig. 6. SEM images a) NFDM before, b) NFDM after, c) WMP before, d) WMP after SHS treatment at 161 ± 1 °C for 60 s.

more easily removed following treatment whereas the high lactose milk powders were more difficult to remove. Allergen residues remained on surfaces following SHS treatment of both food residues. Overall, these results suggest the potential application of SHS in dry food manufacturing environments as a sanitization treatment that compliments but does not replace cleaning. However, additional pilot-scale studies assessing differences in energetic capacities between SSH and other comparable dry sanitation methods as well as assessments of thermal distribution across large surfaces to further the applicability of this technology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2022.109838.

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