



Determination of thermal inactivation parameters of *Salmonella* in non-fat dry milk and hydrated non-fat milk.

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Why Salmonella?

- In the U.S., 1.2 million illnesses with 23,000 hospitalizations and 450 deaths annually (FDA, 2019)
- Infants, elderly and immuno-compromised individuals are at high risk
- The infectious dose for *Salmonella* infection is <1,000 cells, but can be as low as 1 cell (Pal et al., 2016)
- *Salmonella* is responsible for the most number of bacterial foodborne infections (Dewey-Mattia et al., 2018)



Salmonella

- *Enterobacteriaceae* family
- Gram-negative, rod-shaped bacilli
- Includes two species:
 - *S. enterica*
 - *S. bongori*
- *S. enterica* can be further divided into six subspecies using their phenotypic profile
- *S. enterica* ssp. *enterica* serovars can be associated with human infections



Concern in nonfat dry milk (NFDM)

- Used as an ingredient in many foods, especially infant formula
- The incidence of salmonellosis among infants is higher compared to other age groups
- Post pasteurization contamination of milk can occur
- Presence of *Salmonella*, even in low numbers in low water activity (a_w) foods such as NFDM can pose a significant health risk
- Causes severe enteric (typhoid) fever and enterocolitis



Research objective

- To determine the survivability of *Salmonella* in NFDM and hydrated NFDM during 90 days of storage
- To determine the effect of extended storage of NFDM on the thermal resistance (D- and z-values) of *Salmonella*



Experimental Design

- This study was designed as a randomized complete block with repeated measures, with three replications as blocks
- The linear regression graphs for calculating D- and z-values were plotted using Microsoft Excel 2019
- Statistical differences were calculated using ANOVA at $P \leq 0.05$ using SAS® University Edition



Materials and methods

Salmonella enterica subsp. *enterica* serovars used in the study:

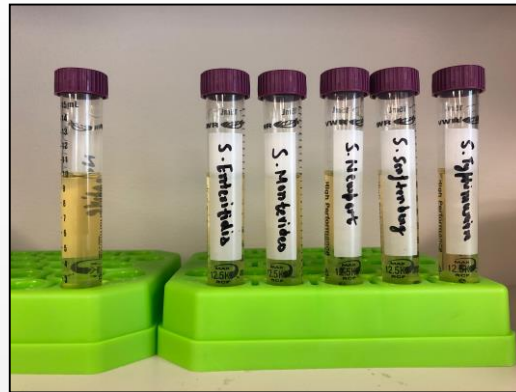
- Enteritidis (ATCC® BAA-708)
- Montevideo (ATCC® BAA-710)
- Newport (ATCC® 6962)
- Senftenberg 775W (ATCC® 43845)
- Typhimurium (ATCC® 14028)



Salmonella culture propagation and inoculum preparation

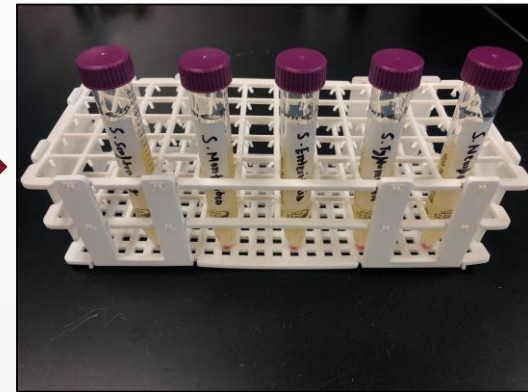


Frozen beads



10 mL BHI broth

37°C/24 hrs.

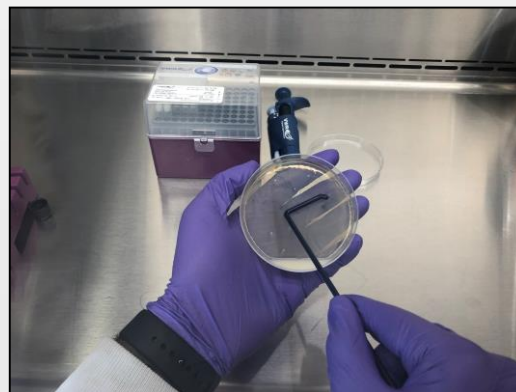


Propagated cultures

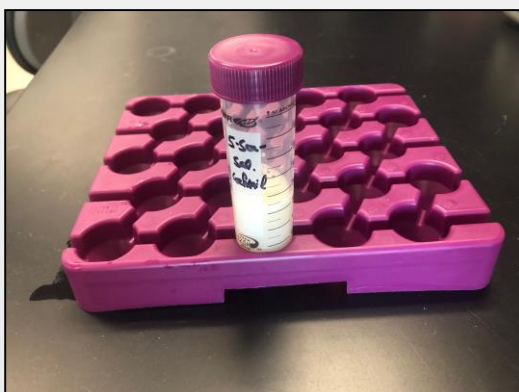
37°C/24 hrs.



Bacterial lawns



Harvesting the lawns

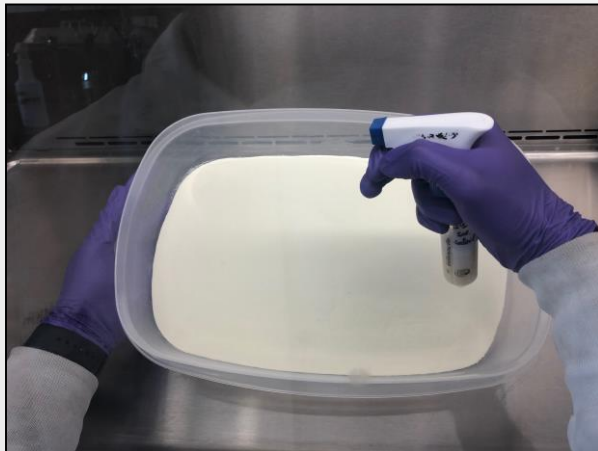


Master inoculum



Non fat dry milk inoculation

- NFDM was mist inoculated and dried back to original pre-inoculation weight at 37°C to achieve ~8 log CFU/g *Salmonella* in NFDM
- The inoculated NFDM was sealed with airtight lids and stored at room temperature (~25°C)



Mist inoculation of NFDM

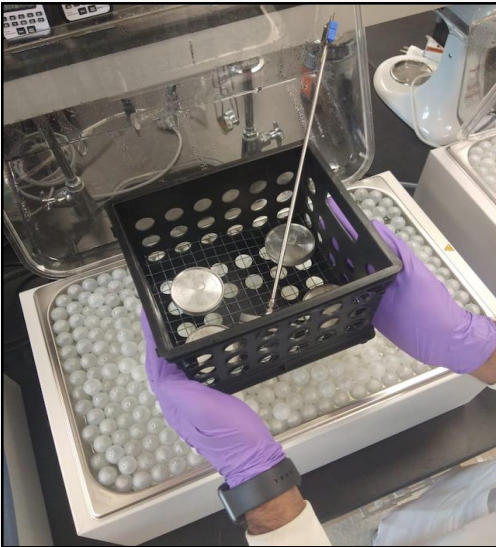


Drying of NFDM



Thermal treatments

- D- values were determined at 80, 85 and 90°C for NFDM; and 59, 62 and 65°C for hydrated NFDM



Immersing TDT discs
in hot water bath



Transferring treated TDT
discs to ice water bath



Thermocouples
monitoring the product
and water temperature



Heat treatments

	D-value Temp. (°C)	Sampling time (minutes)
Nonfat dry milk (NFDM)	80	14
	85	7
	90	3.5
Hydrated NFDM	59	3
	62	1.5
	65	0.5

Sampling times used for calculating D-values at indicated temperatures



Sampling and enumeration

- Heat treated NFDM or hydrated NFDM were transferred to stomacher bags, and then serially diluted using 0.1% peptone water solution
- *Salmonella* was enumerated using **injury recovery media** i.e. brain heart infusion (BHI) agar overlaid with xylose lysine deoxycholate (XLD) agar, and incubated at 37°C for 24 hours



Culture confirmation

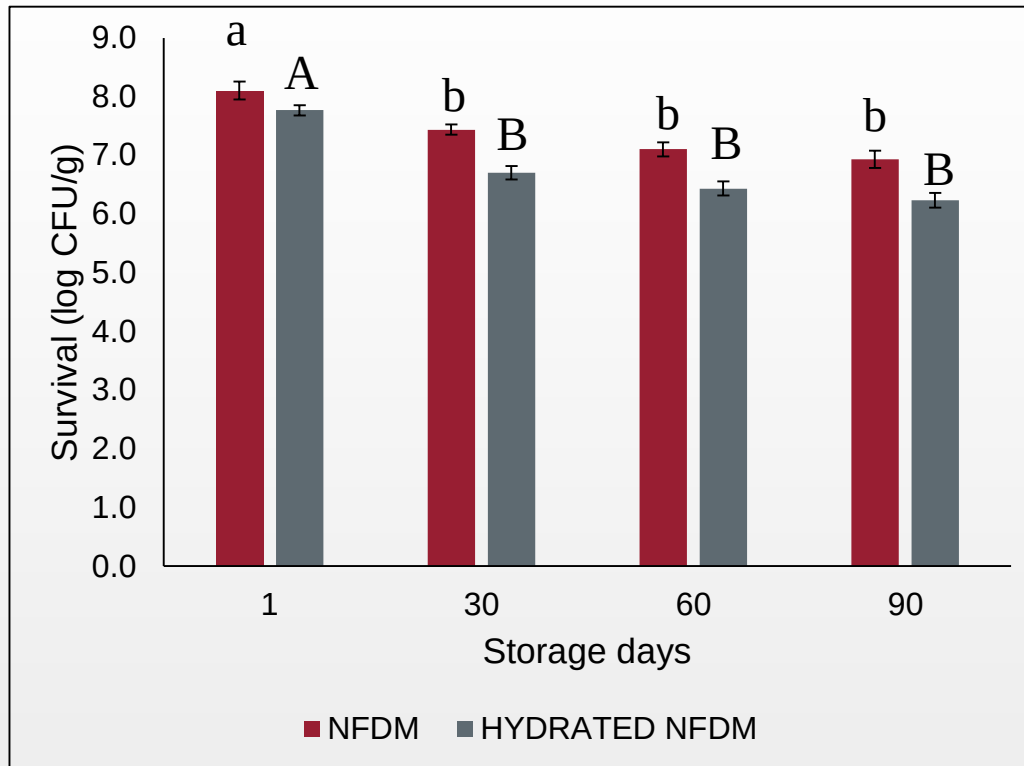
- All cultures were confirmed using API 20E before starting the experiment
- Colonies from NFDM and hydrated NFDM on enumeration media were also confirmed using API 20E



API confirmation



Salmonella survival



Survival of 5- serovar *Salmonella* in NFDM and hydrated NFDM on the indicated days



Salmonella D-values in NFDM

Storage day	80°C	85°C	90°C	z-value
1	17.9 ± 0.33 ^a	9.1 ± 0.25 ^a	4.4 ± 0.09 ^a	16.3 ± 0.30 ^a
30	18.5 ± 0.87 ^a	9.0 ± 0.49 ^a	4.3 ± 0.27 ^a	16.0 ± 0.16 ^a
60	18.6 ± 0.09 ^a	9.1 ± 0.26 ^a	4.5 ± 0.39 ^a	16.4 ± 1.07 ^a
90	18.8 ± 0.33 ^a	9.1 ± 0.27 ^a	4.8 ± 0.17 ^a	16.9 ± 0.04 ^a

The D-(min) and z-values (°C) (mean ± SE) of 5-serovar *Salmonella* cocktail in nonfat dry milk on indicated temperatures and days



Salmonella D-values in hydrated NFDM

Storage day	59°C	62°C	65°C	z-value
1	5.7 ± 0.33 ^a	2.3 ± 0.12 ^a	0.6 ± 0.09 ^a	6.4 ± 0.18 ^a
30	6.1 ± 0.19 ^a	2.3 ± 0.14 ^a	0.6 ± 0.27 ^a	6.1 ± 0.04 ^a
60	6.3 ± 0.21 ^a	2.4 ± 0.15 ^a	0.7 ± 0.39 ^a	6.2 ± 0.35 ^a
90	6.4 ± 0.25 ^a	2.5 ± 0.12 ^a	0.7 ± 0.17 ^a	6.2 ± 0.10 ^a

The D-(min) and z-values (°C) (mean ± SE) of 5-serovar *Salmonella* cocktail in hydrated nonfat dry milk on indicated temperatures and days



pH and a_w

Storage day	NFDM	Hydrated NFDM	Water used for hydration
1	6.62 ± 0.03^a	6.53 ± 0.02^a	5.50 ± 0.15^a
30	5.30 ± 0.01^b	6.53 ± 0.01^a	5.84 ± 0.31^a
60	5.46 ± 0.07^b	6.49 ± 0.02^a	6.84 ± 0.37^a
90	5.30 ± 0.02^b	6.60 ± 0.03^a	6.39 ± 0.58^a

pH of nonfat dry milk (NFDM), hydrated NFDM and water used for hydration on the indicated storage days

Storage day	a_w
1	0.20 ± 0.0^a
30	0.23 ± 0.01^b
60	0.23 ± 0.01^b
90	0.25 ± 0.0^b

a_w of nonfat dry milk on indicated storage days



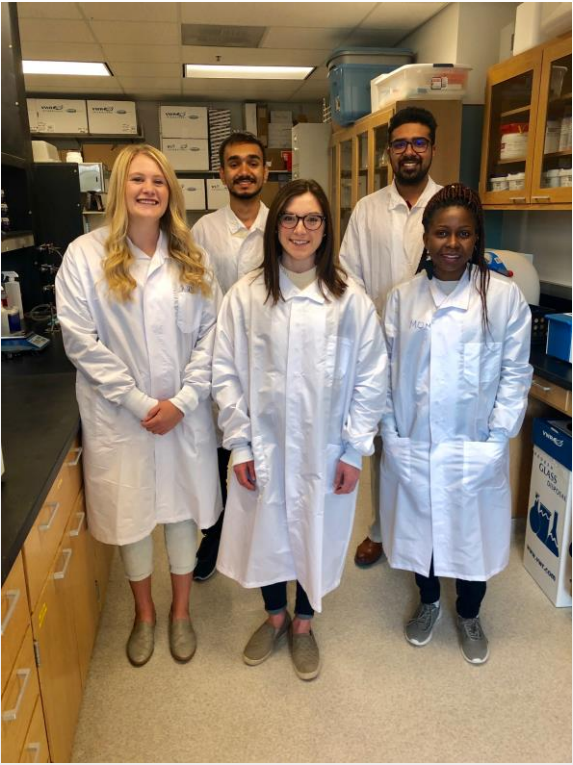
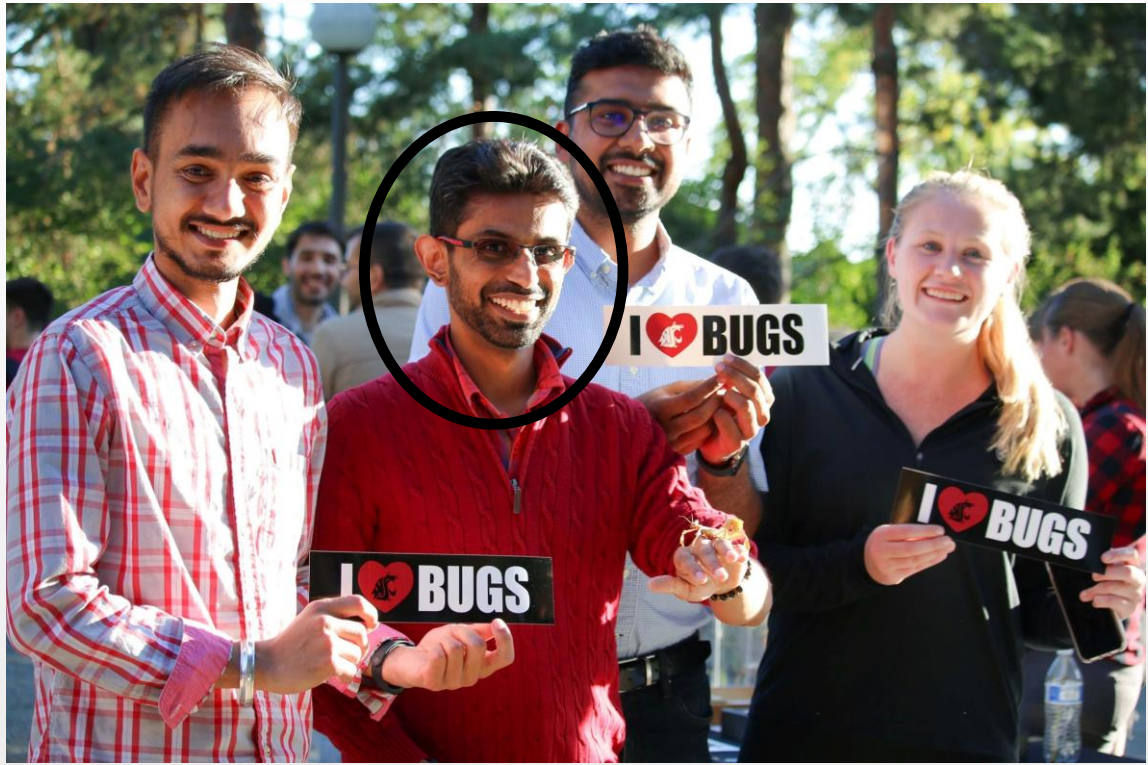
Conclusions

- Contrary to common expectation, *Salmonella* heat resistance did not increase during the dry storage of 90 days
- This study will be continued to determine the changes in thermal resistance due to longer storage (up to one year)
- Consumers should take extra care in storing the NFDM to avoid *Salmonella* contamination
- To study the effect of fat on the survivability and thermal resistance of *Salmonella*, an extended storage study using whole milk powder is also under progress



References

- Dewey-Mattia, D., Manikonda, K., Hall, A. J., Wise, M. E., & Crowe, S. J. (2018). Surveillance for foodborne disease outbreaks United States, 2009–2015. *MMWR Surveillance Summaries*, 67(10), 1–11.
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- Pal, M., Alemu, J., Mulu, S., Parmar, O. K. B. C., & Nayak, J. B. (2016). Microbial and hygienic aspects of dry milk powder. *Beverage & Food World*, 43(2), 28–31.





Thank you

